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EVISCERATION OF FROZEN, DEFROSTED, DRESSED POULTRY

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The principal advantage to be gained in eviscerating poultry is that, if correctly handled, poultry can be placed in trade channels free of visceral taints. If quality has been impaired by visceral taints before the birds were eviscerated, then it becomes obvious that a main objective to be realized by employing the method has been defeated.

Adequate procedures to follow when eviscerating unfrozen dressed poultry have been fairly well established. This poultry may be eviscerated immediately after being defeathered (warm evisceration) or it may be effectively chilled and then eviscerated promptly. Even in instances where poultry is held near 0°C.(32°F.) evisceration should not be delayed longer than several days if quality is to be satisfactorily maintained.

Adequate methods for handling dressed frozen poultry intended for subsequent defrosting and evisceration have not been so clearly defined. Stewart, Hanson, and Lowe (1943) froze dressed birds with a brine spray within five hours after dressing. After holding this poultry for as long as six months in freezer storage, it was defrosted, eviscerated, and compared organoleptically with birds eviscerated 18 hours after dressing and stored for the same length of time. It was found that the frozen, defrosted, eviscerated birds so handled were superior in quality to the birds eviscerated 18 hours after dressing. Trelease and Koonz (1945) found that poultry which was ice-slush-chilled and placed in a freezer three hours after dressing and eviscerated at once upon becoming defrosted compared favorably organoleptically with warm eviscerated poultry.

The purpose of the present investigation was to determine the quality of cooked poultry derived from dressed birds placed directly in the freezer without being chilled in the conventional manner. When dressed poultry is handled in this manner, chilling and freezing become one continuous procedure.

EXPERIMENTAL METHODS

The birds used in the study were White Plymouth Rocks of heavy fryer size. All birds had been similarly fed and otherwise handled from day-old chicks. On the day they were dressed, each bird was individually fed and was dispatched about six hours later, by which time the crop was relatively empty. Poultry was semihard-scalded and the other conventional dressing procedures were followed. The freezing temperature employed was —28.9°C.(—20°F.). The frozen dressed birds were defrosted rapidly in water before being eviscerated. Prior to cooking, the eviscerated birds were held for 24 hours at 10°C.(50°F.) to permit them to defrost. The birds were cooked by roasting at 163°C.(325.4°F.). They

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were considered cooked when the interior tissues of the thigh reached a temperature of 88°C.(190.4°F.).

Eight birds were handled according to each of the following methods:

(a) Poultry placed in freezer at once after removal of feathers, eviscerated immediately upon becoming defrosted, refrozen, defrosted, cooked.

(b) Poultry placed in freezer at once after removal of feathers, immediately upon becoming defrosted placed in a 10°C. cooler for 24 hours, eviscerated, refrozen, defrosted, cooked.

(c) Air-chilled 24 hours at 2.2°C.(36°F.), frozen, eviscerated at once upon becoming defrosted, refrozen, defrosted, cooked.

(d) Air-chilled 24 hours at 2.2°C., frozen, immediately upon becoming defrosted placed in a 10°C. cooler for 24 hours, eviscerated, refrozen, defrosted, cooked.

The cooked birds were inspected by a committee of three for general aroma and flavor of the dark and white meat. Four birds were inspected each time, one bird being identified with each method of handling. A total of eight such inspections was made. The identity of the birds was not known to the inspectors who were requested to score the aroma of each bird as being (5) very desirable, (4) desirable, (3) slightly desirable, (2) slightly undesirable, and (1) undesirable. The flavor of the dark and white meat was separately scored as being (5) excellent, (4) very good, (3) good, (2) fair, and (1) poor.

TABLE 1

Average Aroma and Flavor Scores for Cooked Poultry Handled in Accordance With Four Different Methods

Method of handling poultry	Average scores		
	Aroma	Flavor of dark meat	Flavor of white meat
Placed in freezer at once after removal of feathers, eviscerated immediately upon becoming defrosted, refrozen, defrosted, cooked	4.25	4.38	4.26
Placed in freezer at once after removal of feathers, immediately upon becoming defrosted placed in 10°C.(50°F.) cooler for 24 hours, eviscerated, refrozen, defrosted, cooked................	3.79	3.96	3.75
Air-chilled 24 hours at 2.2°C.(36°F.), frozen, eviscerated at once upon becoming defrosted, refrozen, defrosted, cooked....	3.42	3.29	3.88
Air-chilled 24 hours at 2.2°C.(36°F.), frozen, immediately upon becoming defrosted placed in 10°C.(50°F.) cooler for 24 hours, eviscerated, refrozen, defrosted, cooked......................	2.42	2.46	3.21

EXPERIMENTAL RESULTS

Arithmetic averages for aroma and flavor scores for poultry handled in accordance with each of the four methods (Table 1) indicate that the quality of the poultry placed in the freezer at once after removal of feathers and eviscerated immediately upon becoming defrosted, was superior to that of poultry handled in accordance with the other methods.

Poultry placed directly in the freezer after defeathering and which, after defrosting, was held for one day at 10°C. before being eviscerated was similar organoleptically to poultry air-chilled 24 hours at 2.2°C. prior to freezing and eviscerated at once after being rapidly defrosted. Quality was seriously impaired when birds air-chilled for a 24-hour period prior to freezing were rapidly defrosted and held for one day at 10°C. before being eviscerated.

The results obtained demonstrate the value of rapidly chilling dressed poultry. The temperature of the birds placed directly in the —28.9°C. freezer at once after dressing was lowered from 37.8°C.(100°F.) to about —12.2°C.(10°F.) in 15 hours and a freezing temperature was approached within five hours. Consequently, the time required to chill the poultry or to lower the temperature from 37.8°C. to about —1.1°C.(30°F.) was about the same as the time required to chill poultry by the ice-slush method.

Although quality was very successfully maintained for "warm frozen" poultry, it should be pointed out that entirely different results may be anticipated if chilling and freezing are not accomplished under the rather ideal conditions of this experiment. To employ this method successfully, the freezer should carry a low temperature and should have sufficient capacity so that the low temperature will be effectively maintained when additional freshly dressed, warm poultry is being introduced.

The results of the present study demonstrate very clearly the value of eviscerating dressed poultry promptly after it has become defrosted. This was particularly in evidence for eviscerated poultry derived from dressed poultry which had been air-chilled, frozen, and held at 10°C. for 24 hours after being rapidly water-defrosted. This poultry was distinctly inferior in quality to poultry handled similarly except that it was eviscerated at once after becoming defrosted. Also, the quality of this poultry was inferior to that of poultry rapidly chilled by being placed in the —28.9°C. freezer promptly after dressing and which, after being water-defrosted, was placed in the 10°C. cooler for 24 hours. Again the value of prompt chilling becomes apparent in that it tends to protect quality in the event that the poultry later is subjected to adverse holding conditions.

SUMMARY

The quality of eviscerated poultry obtained from dressed birds placed directly in the freezer without first being chilled in a conventional manner proved to be very good.

The value of eviscerating frozen dressed poultry promptly after defrosting has been demonstrated.

Chilling poultry rapidly and to a low temperature tends to protect quality in the event that birds later are subjected to adverse holding conditions.

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INFLUENCE OF MINERAL LEVELS UPON CAROTENE AND ASCORBIC ACID CONTENTS OF SWISS CHARD GROWN IN THE GREENHOUSE

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Emphasis upon production of high-vitamin foods has focused much attention upon the effect of environmental conditions, including fertilizers, upon the vitamin content of vegetables. The effects of soil-fertility levels on the vitamin content of vegetables have been well summarized by Maynard and Beeson (1943); consequently, only a few closely related researches will be reviewed here.

Isgur and Fellers (1937) investigated the effect of different levels of nitrogen, phosphorus, and potassium upon the vitamin C content of New Zealand spinach and Swiss chard, and found higher ascorbic acid content in Swiss chard at the higher nitrogen levels. No constant differences were found in New Zealand spinach.

Ijdo (1936) reported that spinach grown in sand had a slightly lower ascorbic acid content when the nutrient solutions were deficient in nitrogen, calcium, and magnesium and that it had a considerably higher content when grown on a potassium-deficient solution. Carotene content was slightly lower for plants grown in the calcium-deficient solution, slightly higher for those in the magnesium-deficient solution, much lower in the nitrogen-deficient solution, and much higher in the potassium-deficient solution. Higher levels of nitrogen produced plants having greater carotene and ascorbic acid contents; higher potassium produced higher ascorbic acid and lower carotene contents; while phosphorus seemed to have no effect.

On the other hand, VonHahn and Görbing (1933) reported that the use of a well-balanced fertilizer produced spinach with the highest ascorbic acid content and that excess nitrogen caused a decrease of ascorbic acid. Reder, Ascham, and Eheart (1943) found that nitrogen and potassium fertilizer caused significant decreases in the ascorbic acid content of turnip greens; while phosphorus and calcium fertilizers caused no significant changes in this respect. Wittemore (1934) and Scheunert and Wagner (1939) found the use of manganese, phosphorus, nitrogen, and potassium on soils low in these elements did not increase the vitamin A potency of several leafy vegetables.

Since the design of most of these experiments did not permit the use of statistical analysis and since in many cases the conclusions were based on only a few determinations, it is perhaps not surprising that many experi-

mental results are contradictory to one another. The following experiment
was conducted with the hope of clarifying some of these contradictions.

EXPERIMENTAL PROCEDURE

Seeds of Lucullus Swiss chard were sown directly in greenhouse plots
(two by four feet) containing cinders, "Haydite" (burnt shale), or gravel.
The plots held 10 gallons of solution which was pumped up into the plots
twice daily. Solutions used in deficiency plots were made up with distilled
water; while tap water was used for the complete fertilizer plots. Solutions
for the complete plot contained the following:

Nutrient	Concentration	Source
	$p.p.m.$	
Calcium	200	$Ca(NO_3)_2 \cdot 4H_2O$, $Ca(H_2PO_4)_2 \cdot H_2O$, $CaCl_2$
Nitrogen	150	$Ca(NO_3)_2 \cdot 4H_2O$
Potassium	150	KCl
Phosphorus	50	$Ca(H_2PO_4)_2 \cdot H_2O$
Magnesium	25	$MgSO_4$
Iron	5	$FeSO_4$
Boron	1	H_3BO_3
Manganese	1	$MnSO_4 \cdot 4H_2O$
Copper	0.1	$CuSO_4 \cdot 5H_2O$
Zinc	0.1	$ZnSO_4 \cdot 7H_2O$

For the deficient plots the same compounds were used except that
compounds containing the element to be made deficient were omitted. For
the calcium-deficient plots potassium nitrate was used in place of calcium
nitrate and potassium dihydrogen phosphate in place of calcium hypo-
phosphate. For plots containing an excess of one nutrient the same chemi-
cals were used, but double amounts of the element in question were added
to the nutrient solutions; solutions of the nutrient were checked every
two weeks and replenished to their original strength. Nitrates were tested
according to Spurway (1938) and the other elements, according to Morgan
(1935). The hydrogen-ion concentration was maintained between pH 6
and pH 7 by using a 10-per cent hydrochloric or sulfuric acid to lower
the pH and a 10-per cent sodium hydroxide solution to raise it.

The plants were grown during the spring of 1945 in the deficiency
plots until deficiency symptoms began to appear. In the case of calcium,
no deficiency symptoms appeared; in the case of manganese and magne-
sium, only slight or questionable symptoms appeared. However, these plots
were sampled in the same manner as the others. Samples for carotene and
ascorbic acid determinations were then taken simultaneously while the
plants were still in a marketable condition (before marked symptoms
occurred). The samples were always taken between 8 and 9 A. M., and
entire plants, with the exception of the growing tip and the next youngest
leaf, were taken for samples. As a rule, only one plant was taken as a
sample; however, where much stunting occurred, several entire plants
were taken and a composite sample was made. These samples were coarsely
chopped with scissors and mixed as thoroughly as possible and were then
subsampled for carotene and ascorbic acid determinations. Ascorbic acid
was determined by a modified Morell (1941) method, using an Evelyn

photoelectric colorimeter. Carotene was determined by a modified Moore and Ely (1941) method, using a Coleman Model 11 spectrophotometer.

RESULTS

Average results for carotene contents of the plots are recorded (Table 1) and average results of the ascorbic acid contents (Table 2). The original data were treated to an analysis of variance by the methods of Snedecor (1940). F values were obtained by dividing the mean square for the variation between plots by the mean square for the variation within plots. By this test the variance among plots was found to be highly significant for both carotene and ascorbic acid. Results from each individual plot were then compared with the complete plot by the same method. F values obtained from this comparison (Tables 1 and 2) make it apparent that chard grown on the nitrogen-, iron-, and magnesium-deficient plots was significantly lower in carotene when compared with that from the complete plot. Plants from the calcium-, magnesium-, potassium-, and phosphorus-deficient plots did not vary significantly from those of the complete plot with respect to carotene. With the exception of the double-nitrogen plots, insufficient data regarding carotene were obtained for statistical treatment of the excess-nutrient plots; the former is obviously not significantly different from that of the complete plots.

The ascorbic acid contents of chard grown in the nitrogen-, magnesium-, potassium-, and manganese-deficient plots were significantly greater than that obtained from the complete plot. Plants grown on the iron-, calcium, and phosphorus-deficient plots were not significantly different from those of the complete plots. The plants receiving double amounts of nitrogen, phosphorus, and potassium were found to be significantly lower in ascorbic acid; while plants receiving double amounts of iron, magnesium, and calcium did not vary significantly in ascorbic acid when compared with those receiving complete fertilizer.

TABLE 1

Carotene Content of Swiss Chard Grown in Mineral-Deficient Solutions and in Presence of Excess Minerals

Plot	Number of duplicate samples	Average carotene content (fresh basis)	F value
		mg./100 gm.	
Complete	18	4.18	......
Nitrogen-deficient	7	2.41	11.84[1]
Iron-deficient	7	2.43	12.09[1]
Magnesium-deficient	8	2.90	5.53[2]
Calcium-deficient	6	3.56	1.50
Manganese-deficient	5	4.01	1.20
Potassium-deficient	7	4.93	2.17
Phosphorus-deficient	7	4.99	2.42
Double-nitrogen	4	4.26	......
Double-magnesium	1	5.86	......
Double-calcium	1	5.96	......
Double-potassium	2	4.51	......
Double-phosphorus	1	5.84	......

[1] Highly significant; F value exceeds one-per cent level.   [2] Significant; F value exceeds five-per cent level.

TABLE 2

Ascorbic Acid Content of Swiss Chard Grown in Mineral-Deficient Solutions and in Presence of Excess Minerals

Plot	Number of duplicate samples	Ascorbic acid content (fresh basis)	F value
		mg./100 gm.	
Complete..	22	24.6	......
Nitrogen-deficient................................	7	31.7	7.72[1]
Iron-deficient.......................................	7	24.4	......
Magnesium-deficient............................	9	34.9	13.19[1]
Calcium-deficient.................................	7	29.1	2.62
Manganese-deficient............................	5	31.0	5.76[2]
Potassium-deficient.............................	8	37.5	19.33[1]
Phosphorus-deficient...........................	7	29.3	3.46
Double-nitrogen..................................	5	16.2	8.95[1]
Double-iron..	3	19.6	2.16
Double-magnesium..............................	3	23.9	......
Double-calcium...................................	3	23.1	......
Double-potassium...............................	4	18.0	5.13[2]
Double-phosphorus..............................	3	11.2	15.71[1]

[1] Highly significant; F value exceeds one-per cent level.   [2] Significant; F value exceeds five-per cent level.

DISCUSSION

The data obtained indicate that the carotene content of chard may be decreased by nitrogen, iron, and magnesium deficiencies. The data obtained concerning the effect of excessive amounts of minerals upon carotene content were insufficient to draw any definite conclusions; however, these data suggest that carotene may be increased by excessive amounts of minerals.

The ascorbic acid content of chard is increased by low levels of nitrogen, magnesium, manganese, and potassium. Conversely, large amounts of nitrogen, potassium, and phosphorus decreased the ascorbic acid content. No yield records were taken in the greenhouse. It was noted, however, that plants were greatly stunted on the nitrogen- and potassium-deficient plots and somewhat stunted on the magnesium- and manganese-deficient plots. Little or no stunting was noted in the other plots. For an indication of possible yields, Table 3 is included; in this table the yields for chard

TABLE 3

Yields of Swiss Chard (Pounds per 70-Foot Row) Grown in Field With Different Levels of Fertility

Plot	Variety		Total
	Lucullus	Large Green White Ribbed	
Check (no fertilizer for 16 years).............................	281	194	475
8-20-12 (1,000 pounds per acre).............................	311	268	579
0-20-12 (1,000 pounds per acre).............................	160	100	260
8- 0-12 (1,000 pounds per acre).............................	278	221	499
8-20- 0 (1,000 pounds per acre).............................	235	232	467
16-20-12 (1,000 pounds per acre).............................	493	367	860
8-40-12 (1,000 pounds per acre).............................	299	295	594
8-20-24 (1,000 pounds per acre).............................	523	307	830

grown on the Ohio Agricultural Experiment Station plots in 1945 are recorded. It is noteworthy that the yields from the plots receiving no additional nitrogen are decidedly lower for both varieties than those obtained from the plot receiving a complete fertilizer. The difference between the plot receiving no phosphorus and the plot receiving a complete fertilizer is less marked.

An excess of nitrogen and potassium greatly increased the yields, though an excess of phosphorus had little effect upon the yield. From all of these data one might conclude that the increases in ascorbic acid content under different fertilizer treatment may be due to decreased yield and size of plants, and conversely, an increased yield and size of plant may result in a lower ascorbic acid content per unit of fresh matter. This would also indicate that synthesis of ascorbic acid by the plant is little impaired by mineral deficiencies.

Failure to obtain significant differences between the plants grown in calcium-deficient and complete plots cannot be interpreted as indicating that calcium deficiency has no effect upon carotene or ascorbic acid content, since plants in the calcium-deficient plot failed to develop any evidence of calcium deficiency during the course of the experiment. This is also true to a lesser extent for the carotene value obtained from plants grown in the manganese plot where only doubtful symptoms were noted. The data on carotene appear to support the concept that minerals which affect the formation of chlorophyll also affect the formation of carotene present and, consequently, that deficiency which causes a decrease in chlorophyll causes a decrease in carotene. On the other hand, no such relationship appears to exist for ascorbic acid.

SUMMARY AND CONCLUSIONS

The influence of nitrogen, calcium, potassium, phosphorus, magnesium, iron, and manganese deficiencies upon the ascorbic acid and carotene contents of Lucullus Swiss chard was investigated. Significantly lower carotene values were obtained from plants receiving insufficient nitrogen, magnesium, and iron. Significantly higher ascorbic acid values were obtained from plants deficient in nitrogen, magnesium, manganese, and potassium. Ascorbic acid contents obtained from plants grown in plots to which double the amount considered adequate of the element in question was added, were significantly lower in the case of the double-nitrogen, double-potassium, and double-phosphorus plots.

Under the conditions of this experiment calcium, manganese, potassium, and phosphorus had no significant effect upon the carotene content of chard, while iron and calcium did not significantly affect the ascorbic acid content of the plants.

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RELATION OF HISTOLOGICAL CHARACTERISTICS TO TEXTURE IN SEED COATS OF PEAS

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One of the most marketable of the commercially frozen vegetables is shelled peas. When proper care is taken in handling, both before and after processing, a good commodity results. Frequently, however, the peas are overmature and have tough skins and mealy, starchy cotyledons, or they may be immature and lacking in desirable texture and flavor. Toughness and other characteristics of texture are related to the histological composition of the skin tissues, and certain off-flavors that frequently develop are related to the histological immaturity of the tissues and their contents.

Many variables may enter into the determination of the textural characteristics of plant tissues. The most consistent of these probably are the structural and compositional changes of the cell walls and cell contents during growth and maturation, and they may be characterized as *inherent* physical and chemical factors. In addition there are factors that influence the rates of growth and development of tissues and those that may alter the composition of the tissue, such as the toughening of pea skins during delay after harvest. Pretreatments and processing methods also cause many changes in the original condition of the tissues in fresh peas. These various factors seem best described as *environmental*.

In some instances it might be difficult to distinguish an environmental factor from an inherent factor. The tendency for pea skins to toughen after harvest may parallel at least a part of the processes that take place in the "ripening" and hardening of the skin tissues during natural maturation, as indicated by investigations made by Bennett (1940) and Buston (1935), on the compositional changes in the tissues of other plants during growth.

Although many botanists have described the anatomy and development of seed coats and embryos in various species of the Leguminosae, no information has been published on the relationship of cell-wall development to sieve size and texture characteristics of maturity in peas intended for commercial processing. For many years knowledge of the chemical composition of plant cell walls has been very limited. Only recently has any appreciable understanding of the relationships of various cell-wall components to the basic cellulosic matrix developed, Bailey (1940). Lack of suitable techniques and methods to characterize these structures has perpetuated a wide gap between histological information and the results of chemical analyses. Fairly recent adaptations of qualitative chemical methods to histological techniques have provided information which correlates with and can be verified by knowledge of the crystallite orientation of cellulose as revealed by polarized light and x-ray studies by Bailey (1940),

Bailey and Berkley (1942), Bailey and Kerr (1935), Berkley (1939), Kerr and Bailey (1934), Reeve (1946A), and others, as well as by chemical analyses made by Bennett (1940), Buston (1935), Norman (1937), Ott and Ball (1943), and others.

The use of recently developed histological methods in understanding textural changes inherent to the developmental anatomy of the seed coats of peas is discussed in the following paragraphs. First, however, consideration is given to the structural aspects of histological changes during development or maturation.

ANATOMICAL STRUCTURE

Nearly all of the seed coat, or integument, of the pea and the lima bean seed is organized into characteristic tissue layers (Figs. 1-3). The outermost layer, or epidermis, is composed of highly specialized cells known as macrosclereids (Figs. 4-6). This layer is covered with an outer cuticle and, at maturity, the cells possess very characteristic wall thickenings which form an internal fluted structure in the upper portions of each cell (Fig. 4). Underlying this layer is another single-layered, specialized tissue composed of osteosclereids. These are characteristically hourglass-shaped and also have secondary wall thickenings at maturity (Figs. 4-6). It is obvious, however, that this second layer does not form a compact, solid tissue as do the cells of the epidermis.

Loosely arranged parenchyma cells comprise the bulk of the remaining tissues in the skins of shelled peas, but the specialized histological structure of the seed coat in the common garden varieties of beans and peas exists in the two outermost layers (M and O in Figs. 1-6). Other specialized tissue zones exist where these two layers are modified in the regions of the hilum and the strophiole (a zone near the hilum or attachment scar) of the seed. These zones occupy a proportionally small extent of the total skin tissue, and the modifications of the two layers is only of structural nature. Both the macrosclereids and osteosclereids in these zones have walls of essentially the same composition as found in their distribution over the rest of the skin.

No attempt will be made to present a detailed description of the growth and developmental changes of these tissues. The following paragraphs constitute an histological evaluation of the inherent structural causes of texture, with particular attention to certain problems encountered in commercial food processing. Comprehensive histological investigations are reported elsewhere by Reeve (1946 A and B). Further details on these regions in relation to water absorption or retention and seed germination may be found in other published research by Pammel (1899), Hayward (1938), Martin and Watt (1944), Ott and Ball (1943), and Zimmerman (1936).

Obviously, tissue in which such specialized wall development occurs, and particularly a compact and continuous layer such as these macrosclereides, will have a direct structural relationship to texture. Further, as the thickenings develop and assume their characteristic chemical composition during maturation, there will be a corresponding relationship to relative maturity of the peas, as indicated by increased sieve sizes and mechanical measurements of skin texture.

With any given variety from a given locality, histological characteristics at different stages of development during a single growing season conform to the rates of maturation reflected by different harvest periods. With sieve size as a general index of development, it is evident that the thickenings become more pronounced during growth (Figs. 7-18).

It is obvious that some peas passing through the sieve will be either younger or older in tissue development than the average for that size. Within a single size the observable degree of cell-wall development was found to be more or less pronounced. Also, the observable degree of cell-wall development was found to be sufficiently variable that the histologically more mature peas of one size frequently showed a more pronounced cell-wall development than the younger peas of the next larger size. These variables are reflected in mechanical measurements of skin texture; for example, Boggs, Campbell, and Schwartze (1942) show considerable range in penetration values obtained for the skins of three varieties of peas within each sieve size, and also show an overlapping of values between succeeding sizes (their Tables 1 and 2).

Over the entire range of development it can be seen that the wall thickenings of the macrosclereides become very pronounced (Figs. 7-18). In the fully mature and ripened seed, with a hardened and resistant integument, the characteristic thickenings of these cells are increased to such an extent that, except for the basal ends (Figs. 15-18), the cell cavities are nearly filled. In contrast, at very early stages of growth the thickenings are not evident or have already begun to form as tiny strands of corrugations along the original thin walls. Before harvest size is reached by the ovules or seeds in the pod, this epidermal layer begins as a compact embryonic tissue of cubical cells which undergo considerable elongation prior to pronounced wall thickening. Botanically, the formation and developmental changes of this macrosclereid tissue form an excellent example of tissue specialization involving cell-wall structure.

Quite naturally, variation exists between different varieties with respect to sieve size and histological maturation. Characteristically small-seeded varieties (such as the Alaska and Early Sweet) show a more pronounced development of macrosclereids at sieve sizes No. 3 and 4 than do medium and larger seeded varieties (such as Thomas Laxton and Teton). Any attempts to give these varietal differences statistical significance by meas-

FIGS. 1-6. Photomicrographs of sections cut radially through the seed coats of peas and lima beans (longitudinal to macrosclereids). Fig. 1—Young Alaska variety pea (about sieve size No. 2), × 240. FIG. 2—Young Fordhook lima bean, × 140. FIG. 3—Older Fordhook lima bean, × 140, showing a portion of a cotyledon. Note that total skin thickness decreases with maturity. FIG. 4—Macrosclereid and osteosclereid layers of mature Laxton's Progress variety pea (about sieve size No. 7), × 550. FIG. 5—Macrosclereids and osteosclereids of young Canner King variety pea (about sieve size No. 3), × 550. FIG. 6—Macrosclereids and osteosclereids of older Canner King variety pea (about sieve size No. 5), × 550. Note how total thickness of these two layers decreases with maturity.

E.C. = epidermis of the cotyledon; FVB = fibrovascular bundle; f = fluted wall thickenings; l.l. = "light line"; M = macrosclereids; m.l. = middle lamellae; O = osteosclereids; p = protoplasmic content of cell cavity; pa = parenchyma tissues; S = starch grains (chloroplasts also present in parenchyma not distinguishable here); W = cell wall. The numbered lines in Fig. 5 refer to the approximate levels of transverse sections shown in Figs. 7-10.
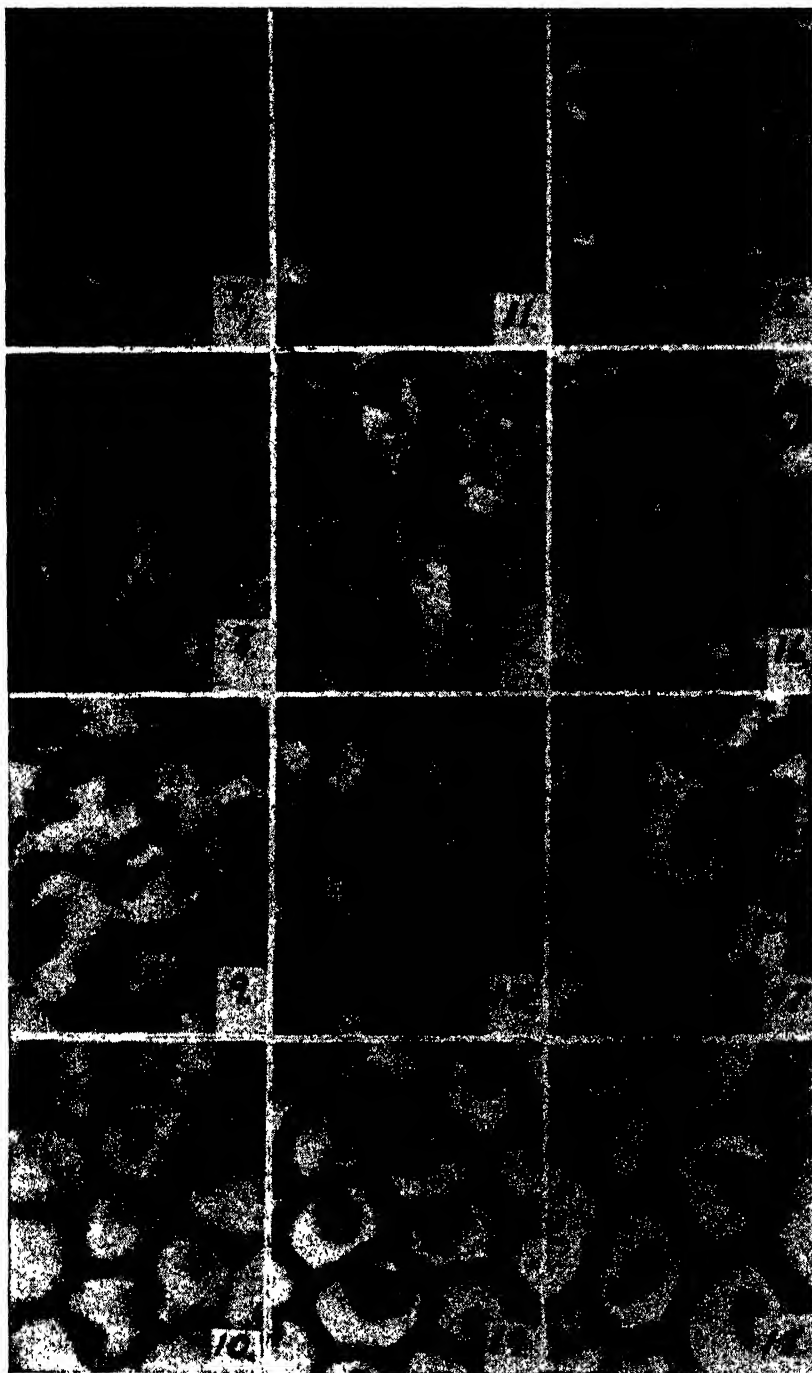
urement of the cell-wall thickenings, however, would be futile because the thickenings are twisted or slightly spiraled along the inside of the cells and, further, lack uniformity of width. Thus, there is no constant which permits statistical evaluation of their visible structural development in either longitudinal or transverse sections of these cells. The variation within a given sieve size alone is sufficient to discount the value of such measurements. The more general varietal characteristics of growth habit, average seed size, and colors are well described and illustrated for different principal types by Hedrick (1928) and by Shoemaker and Delwiche (1934).

Another natural and very common variable encountered in these studies, and found correlated with cell-wall thickening, was an increased rate of maturation in skin tissues toward the end of the harvest season. This characteristic was not limited to larger sieve sizes; it was found in peas as small as Sizes 3 and 4, in which the thickened walls of the macrosclereids were as pronounced as in Sizes 5 and 6. This relationship is a result of changing growth conditions in the field. Also, peas in a single pod are not all the same size, although some varieties are more uniform than others. However, in a given pod the normal, well-formed seeds more nearly represent the same age and development, in spite of difference in size, than do the seeds of many pods graded into any given sieve size. Thus the extent of tissue development and specialization is fundamentally related to texture changes during maturation of the seed.

Relationships between size, maturity, and texture have been investigated by analytical as well as physical methods of measurement. Sayre, Willaman, and Kertesz (1931) show close correlations of growth and size with crushing tests on whole peas. As growth proceeds, the percentages of ''mature'' sizes increase and the crushing values increase somewhat for each size. Boggs, Campbell, and Schwartze (1942), using the penetrometer for texture measurements on the seed coats separated from the cotyledons (as well as crushing values on whole peas), show the same relationships. Penetration values for the skins alone are, of course, of more significance to the histological maturation of the tissues than the crushing values, although both reflect the influence of structural development. It is interesting to note (Table 2 of Boggs, Campbell, and Schwartze) that on any two successive harvest dates the penetrometer values obtained for a given sieve size at the second harvest was consistently nearly equal to, or slightly higher than, the values for the next largest sieve size of the first harvest.

FIGS. 7-18. Photomicrographs of transverse sections through the macrosclereids of seed coats in three different stages of development. Fig. 7 is at a level that includes the top surface. Figs. 11 and 15 are approximately at level No. 1 in Fig. 5. Figs. 8, 12, and 16 are at level No. 2 in Fig. 5. Figs. 9, 13, and 17 are at level No. 3 in Fig. 5; and Figs. 10, 14, and 18 are at level No. 4 in Fig. 5.

FIGS. 7-10—A younger stage of development at sieve size No. 2 in the Mardelah variety, × 1,200. Figs. 11-14—An older stage of development at sieve size No. 2, or representative of sieve size No. 3, × 1,200. FIGS. 15-18—Extent of secondary wall thickening found at sieve size No. 5, × 1,200.

C = cell cavity (dark in some, light in others, depending upon stained contents); m.l. = middle lamellae (not in focus in some of the figures); p = shrunken protoplasmic contents; w = secondary wall development (note particularly the increased development of the thickenings).

This observation agrees with the variations in histological maturity that have just been discussed.

It is natural, although other structures are involved, that a relationship between skin structure (cell-wall development) and texture of whole peas should exist as indicated by a comparison of crushing values, reported in the two papers mentioned, with developments in the walls of the macrosclereides. Carbohydrates are the most important components involved in the formation of plant-cell walls. Thus increased starch content in the cotyledons may parallel the development of secondary wall thickenings in the macrosclereids of the seed coat during growth, because in each case the elaboration of structures (starch grains and cell walls), which are basically carbohydrate, is involved. Neilsen (1943) has proposed starch determination as an index of maturity in starchy vegetables.

CARBOHYDRATES IN WALLS OF MACROSCLEREIDES

The presence of "hemicellulosic" pentosans in the fruit pods and seed skins of many species of plants as well as those of the Leguminosae has been recognized for a number of years. Stark and Mahoney (1942) have characterized the so-called "parchment" layer of the pods of snap beans as containing a "hemicellulose" of the xylan-araban type. Buston (1935) has reported pentosan and hexosan values for many plant tissues, including bean pods. In analytical studies on the seed skins of the common garden bean, Ott and Ball (1943) have found an average polyuronide content of 19 per cent and a "true pentosan" content of 21 per cent on a dry-weight basis. Investigations reviewed by Norman (1937) present similar results which are in general agreement as to tissue composition.

When the present studies were undertaken it soon became obvious that the interpretations of previous histological investigations were not in agreement with published analytical data on cell-wall composition. The shortcomings of the histochemical tests commonly used have been discussed in detail elsewhere with histological evaluation of the wall structure of maturing macrosclereids in pea skins, Reeve (1946A). It was found that several characteristic pentose tests could be adapted readily to histological methods, particularly the phloroglucinol-hydrochloric acid test, used by Stark and Mahoney (1942), Bial's orcinol test, and the xylonic acid test for xylose [Bertrand's reaction; see Morrow and Sandstrom (1935) for details]. The results of these tests, in revealing the pentosan nature of the walls of the macrosclereids and differentiating between the pectic components of the middle lamellae and "encrusting" substances of a more "hemicellulosic" character, are in general agreement with published analytical data and with modern interpretations of cell-wall structure as discussed by Norman (1937), Bailey (1940), Bailey and Berkley (1942), and others.

The following points are of significance in the present discussion:

1. The walls of the maturing macrosclereids contain pentosans that naturally increase as the structural thickness increases. These are of the xylan-araban type, common to the seed skins of many species of plants. The so-called "light line" (Figs. 4 and 6) is not a structural entity and is significant only as a refraction property of these walls.

2. The high resistance of the thickened walls to common extracting agents for hemicelluloses indicates a "cellulosan" type of wall structure, Norman (1937). Dried, ground, pea-skin tissues extracted for several hours with four per cent hot sulfuric acid or with four per cent potassium hydroxide, according to various methods, showed but little evidence of macrosclereid tissue maceration (cell separation) in the fragments of the residue; and the thickened walls of the macrosclereids still gave positive reactions to the pentosan tests when viewed microscopically.

3. In addition, the middle lamellae, which are largely pectic in the younger stages of macrosclereid development, gave good indication that "encrusting" substances of pentosan or "hemicellulosic" nature are formed during the maturation of this tissue in peas.

This last point has been studied in greater detail with particular emphasis on the practical aspects of possible tenderization treatments for the seed coats of peas intended for commercial freezing. Sodium hexametaphosphate, sulfites of both potassium and sodium, oxalic acid, ammonium oxalate, and hydrochloric acid were used in gradations of 0.1 per cent aqueous dilutions of 1:100 to 1:1,000. These were applied for two, five, and ten minutes to sections and to dried, ground samples of pea skins. Three methods of application were used as follows: (1) cold solutions, (2) hot solutions, and (3) alternate hot and cold solutions. The preparations were then flushed with distilled water, partially dried on microscope slides, and tested histochemically with the previously described pentosan indicators as well as with stains that differentiate the pectic middle lamellae from the cell walls. Histologically prepared sections of the skin tissues were found to give the same results as were obtained with fresh or previously untreated sections.

No appreciable effect could be observed in the middle lamellae of well-developed macrosclereids. The thickenings themselves were not affected and gave positive pentosan tests, which seemed to be enhanced, possibly by the removal of traces of soluble forms of sugar that might reduce the sensitivity of these tests, Bial's in particular. It was evident that some of the pectic materials of the middle lamellae were removed by the stronger of these treatments, but at this stage of development other "encrusting" substances are present in the middle lamellae and they showed no visible effects when comparisons were made with untreated samples.

In the younger stages (sieve sizes No. 1 and 2) very little "encrusting" material was present and the pectic middle lamellae could be seen to be definitely weakened by the stronger treatments with these reagents. With alternate treatments of ammonium oxalate and hydrochloric acid or other combinations of reagents and flushing with water between applications, cf. Kerr and Bailey (1934), maceration or cell-separation of the macrosclereids could be accomplished while most of the pectic material was removed. This was not easily accomplished, however, in sieve sizes representative of moderately aged and mature peas. It seems very likely, therefore, that the tenderization effects described by Nielsen (1943) involve only changes that reduce the toughening effects of calcium and magnesium on the seed skins. Any chemical treatment capable of altering the pentosan complex of the cell walls and middle lamellae of older peas very likely

would be unsuitable for commercial processing methods where retention of qualities other than texture is necessary.

INFLUENCE OF NUTRITION ON SKIN TEXTURE

Sayre, Willaman, and Kertesz (1931) showed that increases in calcium chloride content of soil resulted in increased toughness of skins in field-grown peas, but application of fertilizers with other calcium salts had no pronounced effect upon skin texture. They also performed water-culture experiments with solutions of adjusted calcium and potassium concentrations. In these there was a correlation between high-calcium nutrition and thick, tough skins. Peas with a low-calcium nutrition had thinner skins and were not as tough as those from plants of high-calcium culture. Increased thickness of skins also was found to be correlated with high-potassium nutrition, but the skins were not so tough as those from plants with low-potassium nutrition and with thin skins.

Histological aspects of these nutritional effects upon texture were investigated in the present studies with six varieties of peas.[1] Nutrient solutions of adjusted calcium and potassium contents were prepared by adding to one liter of distilled water stock solutions of 1 N strength in the amounts listed (Table 1).

TABLE 1
Composition of Nutrient Solutions

Solution	Control	High Ca, low K	Low Ca high K
	ml.	*ml.*	*ml.*
CaNO₃	5	9	1
KNO₃	5	1	9
MgSO₄	2	2	2
KH₂SO₄	1	1	1

One ml. of one-per cent iron tartrate was added to each liter of the prepared nutrient solutions, and the same amount was added weekly with the change to new solution during growth of the plants. In addition, one ml. of the so-called "A to Z" solution, Meyer and Anderson (1939), was supplied to each liter of prepared nutrient solution to insure availability of trace elements. Fifteen to 20 plants of each variety were grown in each of the three solutions, averaging three per gallon jar, and each jar was p᷐ with a mechanical aerator adjusted so that a slow, fine bubbling of air was continuous through the solution. The gallon jars were covered with heavy paper or painted black, to protect the roots from light and to retard growth of algal forms. Growth to harvest was good in nearly all cases. During growth the pH of the solutions remained between 4.5 and 5.0, dropping to that more or less constant level from an original 6.7 to 6.9 within a few hours each time the plants were transferred to new solution. Otherwise the nutritional conditions remained fairly constant.

In the sampling of peas for penetrometer and histological studies, blossoms were tagged so that at harvest all pods of approximately the same

age could be segregated. However, because different varieties mature at different rates, this segregation does not indicate uniformity between varieties. Pods with fewer than four ovules and abnormal pods were discarded. The peas were hand shelled and treated as follows for penetration tests: (1) cooked in flowing steam in an autoclave for 12 minutes, (2) cooled to room temperature and cut in half through the hilum and along the strophiole in order to obtain uniform halves of skins, and (3) tested (skins only) on the Boggs penetrometer, Candee and Boggs (1941). This instrument operates automatically and measures in kilogram fractions the weight required for a blunt needle to penetrate the sample. At the point of penetration electrical contact stops the operating lever so that consistent readings can be taken. Thirty or more readings were taken with each lot of duplicated samples, three thicknesses of skins being used for each penetration measurement; the relationship of penetration values to mineral nutrition is summarized (Table 2).

TABLE 2
Results of Penetrometer Tests

Variety	Control			High Ca, low K			High K, low Ca		
	Mean[1]	SD[2]	SE[3]	Mean[1]	SD	SE	Mean[1]	SD	SE
Tall Alderman	.27	.033	.008	0.66	.235	.054	.25	.032	.01
Pride	.34	.08	.025	0.44	.071	.018	.32	.046	.01
Laxton's Progress	.68	.15	.009	0.92	.131	.008	.44	.086	.021
Giant Stride	.56	.176	.047	1.04	.34	.098	......	......	......
Dwarf Telephone	.24	.049	.011	0.31	.049	.01	......	......	......
Hundredfold	.76	.09	.02	0.96	.124	.03	......	......	......
Tall Alderman (garden grown)	.416	.174	.030	......	......	......	......	......	......

[1] Mean is expressed in Kg/10. [2] SD = standard deviation. [3] SE = standard error.

Differences in penetrometer values between varieties listed (Table 2) do not indicate varietal differences in texture, because no attempt was made with the small amount of material available to harvest samples at comparable stages of maturity for varietal comparisons. The rates of tissue maturation in these varieties differ sufficiently to make it extremely difficult, if not actually impossible, to obtain corresponding stages of structural development with any degree of certainty.

The solutions used by Sayre, Willaman, and Kertesz (1931) were of different composition from those used here. In their studies thick skins were produced by both high-calcium and high-potassium nutrition, and thin skins by both low-calcium and low-potassium nutrition. Thus it is not clear whether their texture values show relationship to thickness, to cell-wall composition, or to both thickness and composition. In the present studies, the adjustment of calcium and potassium was made so that the same total of these two minerals was available in all three nutrient solutions. When sections cut from comparable areas of the skins were measured microscopically, no appreciable differences in total skin thickness or in wall thickness could be observed. Thus the differences in texture obtained in any individual variety and between samples representing the three

conditions of mineral nutrition used in these studies may be directly correlated with the influence of nutrition upon the materials of the cell walls and middle lamellae.

Mineral nutrition, as it *affects* texture, is essentially an environmental factor, but the *ability to respond* lies within the tissue and involves the materials of tissue structure. Texture change is, then, an inherent histological factor because physiological processes in the formation of the structural materials in cell walls and middle lamellae are involved. Possibly the physical surface behavior of calcium and potassium salts of pectic acids, or some similar relationship of these elements to pectic constituents, would provide a chemical explanation for the effects of mineral nutrition on texture. This explanation would seem logical if Allen's (1901) hypothesis, that the "mature" middle lamellae of plant walls are predominantly calcium pectate, is actual fact. Because many other substances (lignin, "hemicelluloses," and other pectic or polyuronide materials) are characteristic of middle lamellae in plant tissue, and because different sorts of tissue show considerable variation in composition, Kerr and Bailey (1934) prefer to regard the region of the middle lamellae (which are not structural entities as are cell walls) as thin layers of "intercellular substance."

Qualitative histochemical tests for calcium and potassium did not show any significant differences between the skins of peas harvested from plants representing each of the three nutritional conditions which have been described. Several tests, Cowdry (1943), were attempted, but enough of these elements was present in all samples so that no differences could be detected. Further chemical analyses for calcium and potassium in the skin tissues were not attempted because the methods do not permit direct microscopic correlation with structures and materials observable under the microscope. Sayre, Willaman, and Kertesz (1931) list reliable analytical data on the accumulation of these elements in pea skins, but there is no microscopic evidence that the differences are associated with substances of the middle lamellae. This phase of texture problems in the seed coats of beans and peas remains largely hypothetical.

Fundamentally, the characteristics of texture in plant tissues may be divided into two categories: (1) those of living, functioning cells and (2) those of the nonliving, structural components. The substances of the cell walls belong in this second group. Ample demonstrations of the significance of cell-wall components to strength and texture of tissues may be found in textile research made by Berkley (1939) and in studies on wood cells by Bailey and Berkley (1942), Bailey (1940), Bailey and Kerr (1935), and Norman (1937). Differences in the crystallite orientation of cellulose, amounts of such materials as lignin and "hemicelluloses" or pentosans present, and lengths or patterns of molecular chains effect differences in texture and strength in the different kinds of plant tissues and their cell walls. Thus the anatomical structure and development of pea skins, as discussed here, not only involve the growth of wall thickenings during maturation, as previously discussed, but also include quite distinctive submicroscopic characteristics of these specialized walls. These characteristics of submicroscopic structure and chemical composition may be considered the fundamental basis of texture and cause of texture change in pea skins.

DISCUSSION

An attempt has been made to present a more or less natural classification of the causes of texture and texture change in the seed skins of peas. Thus the distinction between "inherent," histological factors and "environmental" factors has been made. In general, the same sort of classification can be applied to any of the vegetables used in commercial food processing. The factors of environment include growing conditions, harvest, vining, handling, and even processing treatments. Published research on texture and related qualities has been confined almost exclusively to investigations concerned with the factors of this second category. These variables, more obvious to the food processor, are naturally of more immediate concern to those engaged in food research.

Histological evaluation of the various tissues, their specializations, and the composition of cell-wall developments has been lacking in food research because plant histologists in general have not taken advantage of an opportunity to broaden the horizons of their fields. Such information should be valuable not only to those more immediately concerned with the problems of processing methods but also to those engaged in genetical development and selection of varieties of more desirable quality characteristics. Thus such information would contribute to the solution of some of the problems encountered in commercial food processing.

It is obvious that the various histological factors causing texture change during maturation of the tissues are not equally important. An arbitrary classification of these can be made for the seed coats of peas, based upon sources of information discussed in this paper, by merely listing the factors in order of decreasing significance as follows: (1) degree of development and chemical composition of the secondary walls of the macrosclereids or epidermal cells, (2) changes in composition of the middle lamellae during growth and maturation of the tissues, (3) degree of development, etc., of the osteosclereids and their middle lamellae, and (4) the more simplified wall structure of the parenchyma tissue and its middle lamelle, and the outer cuticular layer of the skin.

Sayre, Willaman, and Kertesz (1931) cite Lee and Priestley (1924), who advance the theory that soluble potassium "soaps," formed with the fatty acids along the protoplasmic membranes within the cells, migrate to the surface of the skin and thus contribute to formation of the cuticle. In contrast, insoluble "soaps," not migrating, tend to withhold the fatty substances in the tissues and along the inner surfaces of the cell walls. Sayre, Willaman, and Kertesz suggest that this latter condition may cause toughening of the tissues. Priestley (1943) has published a more extensive review, including recent research on the plant cuticle and its development. In the meantime, several investigators have suggested that the mechanism of cuticle formation just described may be used to explain some of the texture changes in the seed coats of beans and peas. Possibly such theoretical considerations may apply to the processes involved in seed germination.

In the writer's opinion, it is misleading to attempt an explanation for changes in skin texture encountered in commercial food processing of peas on the basis of theories concerned with cuticle formation. The cuticle is an

inherent factor of texture, but the development of the thickened secondary walls of the macrosclereids and their chemical composition obviously have more influence on texture, because the cuticle is only a very thin, waxy layer.

Various suggestions have been published about the calcium and magnesium contents of skins during delay after harvest. No really complete analysis has been supplied for comparing changes in tissue composition with data on mineral content during delay or other environmental conditions to which harvested peas may be subjected. Nevertheless, it frequently has been inferred or even accepted that migration of mineral salts from the cotyledons to the skins occurs after harvesting and shelling as a natural process and causes skin toughening.

In the writer's opinion, based upon histological considerations, such changes in mineral composition may be of an apparent nature rather than actual. The skins of pea and bean seeds arise as separate structures, independent of the cotyledons, and there is no physical attachment connecting the embryo with the skin or seed coat in harvested peas. The embryo and its cotyledons grow by utilizing nutrient tissues which are developed in the ovule following fertilization. Migration of mineral solutions and other soluble substances from cotyledons to skin must necessarily be confined, in shelled peas, to the medium of juices freed as the result of mechanical bruising. When peas are vined and podded mechanically, such bruising may be severe. It is possible that these juices affect skin texture under these conditions.

The effects of cellular processes which take place in the tissues after harvest and during delay may closely parallel the conditions or processes of natural cell-wall elaboration. The results of Bennett's (1940) and Buston's (1935) studies on cell-wall components in forage plants show that cell-wall changes in chemical composition take place during growth and maturation processes and also in slow drying or curing processes.

SUMMARY

The major cause of texture change in the seed coats of peas and other legumes during maturation is the formation of a highly specialized, epidermal structure composed of macrosclereid cells. Pronounced wall thickenings of a pentosan-cellulosic composition are developed and cause pronounced toughening and hardness during maturation of the seed.

Other structures are discussed and their relationship to texture is evaluated and classified in order of histological significance as contributing factors to problems of texture. The cuticle is considered to be of minor importance.

The effects of mineral nutrition were studied and it was demonstrated that the resulting texture, which may be independent of total skin thickness, appears to be related to slight changes in pectic materials. Thus cell-wall substances and structures, as determinants of texture characteristics, are affected by nutrition.

Histological characteristics as related to problems of texture in processing methods are discussed.

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A NOTE ON USE OF SCHULTZ AND OLSON LETHAL-RATE PAPER FOR CALCULATION OF THERMAL PROCESSES FOR FOOD PRODUCTS IN TIN CONTAINERS

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Schultz and Olson (1940) described a special co-ordinate or lethal-rate paper for use in thermal-process calculations for food products in tin containers. The method of calculation using this lethal-rate paper was a distinct improvement on the graphical method of Bigelow, Bohart, Richardson, and Ball (1920) but, as described, it imposes limitations on the selection of the temperature scale for the lethality graph. It was shown only how the maximum temperature could be made 250°F. plus or minus multiples of the z value for which the lethal-rate paper was constructed. Each different scale requires the use of a different unit area in making the calculations, and Schultz and Olson provided a formula for determining this unit area only within the limits of the scale application noted.

Actually, there need be no limitation on the choice of the scale for the lethal-rate paper if a suitable unit area is calculated for use with the scale selected. In constructing the lethal-rate paper for a given z value of the thermal death curve, the lethal rates (L) for temperatures in the processing range are calculated with 250°F. represented by $L = 1$. The temperature lines are then drawn at the corresponding L units above the base with the top-line unity. Since the thermal death curve representing the relationship between L and temperature is a straight line on semilog paper, it is apparent that there is a temperature coefficient for L. This would mean that a change of a given number of degrees temperature anywhere in the range multiplies or divides the L by a constant factor so long as z does not change. It is found that $L_{240} = \dfrac{L_{250}}{3.594}$; $L_{230} = \dfrac{L_{250}}{(3.594)^2}$; etc. If then, the top line were to represent 240°F., $L_{230} = \dfrac{L_{240}}{3.594}$; $L_{220} = \dfrac{L_{240}}{(3.594)^2}$; etc. The *change* in L from 240 to 230°F. is the same fraction of the total scale when 240°F. is the top line as the change from 250 to 240°F. when 250°F. is the top line. This is true on down the scale and for any temperature interval. Therefore, with no change in z a given line on the paper is always in the correct position to represent the lethal rate at a certain number of degrees below the temperature selected for the top line.

The unit area to be used with any given selection for the top-line temperature is calculated as follows: The unit area must be an area on the paper such that the product of the ordinate (lethality) and abscissa (minutes) equals unity. The lethal rate of the top line may be calculated with a formula given by Schultz and Olson and the distance from the base

to the top line is measured. The number of minutes to the inch on the linear time scale is noted. Then,

$$\text{unit area (in square inches)} = \frac{d}{m} \cdot \frac{1}{L}$$

where d = the number of inches from the base to the top line,
m = the number of minutes per inch on the time scale,
L = the lethal rate for the temperature selected for the top line (when L = 1 at 250° F.).

This unit area represents a sterilization value (F) of 1. The sterilization value for any length of process is found by measuring the area enclosed by the lethality curve and the base line and dividing by the unit area. Unit areas for a graph of the usual dimensions are given (Table 1).

TABLE 1

Unit Areas for Use With Various Maximum Scale Temperatures When d = 10, m = 10, and z = 18[1]

Degrees F.	Unit area	Degrees F.	Unit area
250	1.000	232	10.00
249	1.136	231	11.36
248	1.292	230	12.92
247	1.468	229	14.68
246	1.668	228	16.68
245	1.896	227	18.96
244	2.154	226	21.54
243	2.448	225	24.48
242	2.782	224	27.82
241	3.162	223	31.62
240	3.594	222	35.94
239	4.084	221	40.84
238	4.642	220	46.42
237	5.276	219	52.76
236	5.994	218	59.94
235	6.814	217	68.14
234	7.742	216	77.42
233	8.798	215	87.98

[1] When d = 20, unit areas are one-half of values given.

This added flexibility in applying the temperature scale to the lethal-rate paper not only permits the attainment of the most manageable area under the curve but also simplifies conversion of data from one retort temperature to another. If a curve is available at one retort temperature, the entire temperature scale may be shifted by the amount it is desired to change the retort temperature. The areas already measured for the original retort temperature are then divided by the unit area required by the new top-line temperature to determine the sterilization value of the process at the new retort temperature. In shifting the scale the initial temperature is, of course, changed by the same amount that the retort temperature was changed and a correction for this may be necessary.

Having the top line of the lethal-rate paper represent 250°F. plus or minus multiples of the z value has the advantage that the unit areas are

convenient numbers permitting easy division, whereas the unit areas are long decimal numbers when other scales are used. Especially when a calculator is available, however, the advantages of free selection of the scale outweigh this inconvenience. This laboratory has found it convenient to have a scale labeled "degrees below maximum scale temperature" placed in one margin of the printed lethal-rate paper as a guide for labeling the graph after a selection has been made for the top line as dictated by the nature of the data to be plotted.

SUMMARY

A method has been described by which the Schultz and Olson lethal-rate paper for calculating thermal processes for food products in tin containers may be used with free choice of the temperature scale by the application of suitable unit areas. The additional flexibility of the scale yields more convenient areas under the lethality curve, and it is shown how the method permits conversion of data to a new retort temperature without the construction of a new curve.

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EFFECT OF FRENCH DRESSINGS, VINEGARS, AND ACETIC ACID ON RATE OF LOSS OF VITAMIN C IN RAW CABBAGE

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In 1936 some experiments with pure vitamin C [1] and vinegars were carried out by the senior author in an attempt to secure information which might help to explain the vitamin C losses which occur in vegetables during pickle making. In the first experiment a comparison was made of vitamin C losses in mixtures of a solution of pure ascorbic acid (in distilled water) with distilled water, cider vinegar (raw and boiled), and five per cent acetic acid. The results (Table 1) show that the immediate loss was highest in the mixtures of ascorbic acid solution with either raw or boiled vinegar, while that in the mixture containing five per cent acetic acid was lowest. After 24 hours at room temperature the mixtures containing vinegars had lost approximately 85 per cent of the vitamin C present at the time of the immediate test, while the one containing five per cent acetic acid had lost only 15 per cent.

TABLE 1

Effect of Cider Vinegar and Five Per Cent Acetic Acid on Vitamin C Loss in a Solution of Pure Ascorbic Acid (Cebione)

Mixtures	Vitamin C in mixture	
	Immediate test	After 24 hours
	mg./50 ml.	*mg./50 ml.*
10 ml. ascorbic acid sol.[1] + 40 ml. distilled water[2]	8.14	5.14
10 ml. ascorbic acid sol.[1] + 40 ml. 5% acetic acid[2]	8.75	7.44
10 ml. ascorbic acid sol.[1] + 40 ml. raw cider vinegar[2]	7.29	1.07
10 ml. ascorbic acid sol.[1] + 40 ml. boiled cider vinegar[2]	6.86	.95

[1] Contents of 0.1-gm. ampul dissolved in 100 ml. of distilled water.   [2] Mixtures kept in glass-stoppered volumetric flasks at room temperature.

A later experiment, Clayton (1940), carried out with mixtures of an eight-per cent acetic acid extract of raw green pepper and five per cent acetic acid, or malt, cider, and white vinegars (adjusted to five per cent acidity), again showed a much slower rate of vitamin C loss in the mixture containing the acetic acid than in those containing the vinegars. Also rate of loss varied somewhat with the different vinegars, the mixture containing the white vinegar showing the lowest loss and the mixture containing the cider vinegar the highest loss.

In continuation of these experiments with vinegars recent studies have been made of vitamin C losses in individual cabbage salads made with French dressing containing different kinds of vinegar (three and five per

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[1] Merck's Cebione.

cent acidity), acetic acid (three and five per cent acidity), or distilled water. Losses in salads made with plain vinegars (three and five per cent acidity), or acetic acid (three and five per cent acidity)[2] also have been studied. Comparisons have been made between the results obtained when the salads were mixed in white enameled bowls with a metal-tined kitchen fork and in glass bowls with a silver fork. The results of these experiments are reported in this paper.

<div align="center">EXPERIMENTAL PROCEDURE</div>

A supply of new early cabbages was bought from a dealer in February, 1945, and kept in cold storage at approximately 1.1°C.(34°F.). Delivery to the laboratory building was made once a week and the cabbages kept in a cool cellar storeroom until used.

Method of Sampling and Analysis for Vitamin C: A different cabbage was used for each day's series of tests. The cabbage was first cut into quarters (down through the core) and a sufficient amount of the tender part of the head was then shredded with a sharp stainless-steel knife. The width of the shreds was about one-eighth inch. The total amount of shredded cabbage was then thoroughly mixed in a large glass bowl and the samples removed from the mixed lot.

For the raw analysis a 50-gram sample was blended for three minutes in a Waring blendor with 200 ml. of three per cent metaphosphoric acid. Two samples of approximately 19 gm. each of the blended mixture were then quickly dipped out of the blendor with an aluminum cream dipper and weighed in previously weighed weighing bottles. The mixture was quantitatively washed out of each bottle with approximately 10 ml. of three per cent metaphosphoric acid into a small mortar (3½ inches in diameter) and ground for two minutes with five gm. of Merck's reagent sea sand. It was then washed from the mortar with about 15 ml. more acid into a 50-ml. centrifuge tube (with constricted neck), stoppered, and centrifuged for 10 minutes. The fairly clear supernatant liquid was poured into a 100-ml. volumetric flask. The residue in the centrifuge tube was extracted twice more with shaking, using approximately 20 ml. of three per cent metaphosphoric acid each time and adding the supernatant liquid to the flask. The extract in the flask was then made up to volume, shaken, filtered through S and S No. 560 fluted paper and titrated with a solution of dichlorobenzenone-indophenol. A five-ml. microburette was used and the indicator was standardized against Merck's Cebione.

The individual salads were made in either white enameled or glass bowls using 50 gm. of cabbage and 10 gm. of French dressing[3] or an equivalent amount of vinegar or acetic acid (3.4 gm.) in each. Mixing was done with either a metal-tined kitchen fork or a silver fork. The bowls were covered with waxed paper and allowed to stand at room temperature [20

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[2] Studies were also made of the effect of adding the seasonings used in plain French dressing to plain cider vinegar (five per cent acidity) or to five per cent acetic acid before mixing with the cabbage.

[3] Recipe for French dressing: ½ cup vinegar (121 gm.), 1 cup Wesson oil (217 gm.), 1½ teaspoons salt, 1½ teaspoons sugar, ¾ teaspoon mustard, ½ teaspoon paprika, ¼ teaspoon onion juice, dash of red pepper, and ⅛ teaspoon Worcestershire sauce.

to 22.5°C. (68 to 72.5°F.)] for the required time intervals. The same method of analysis was used as for the plain raw cabbage, the salads being washed from the bowls into the blendor with 200 ml. of three per cent metaphosphoric acid. The acidity of the vinegars was determined by titration with standard NaOH and necessary dilutions were made with distilled water.
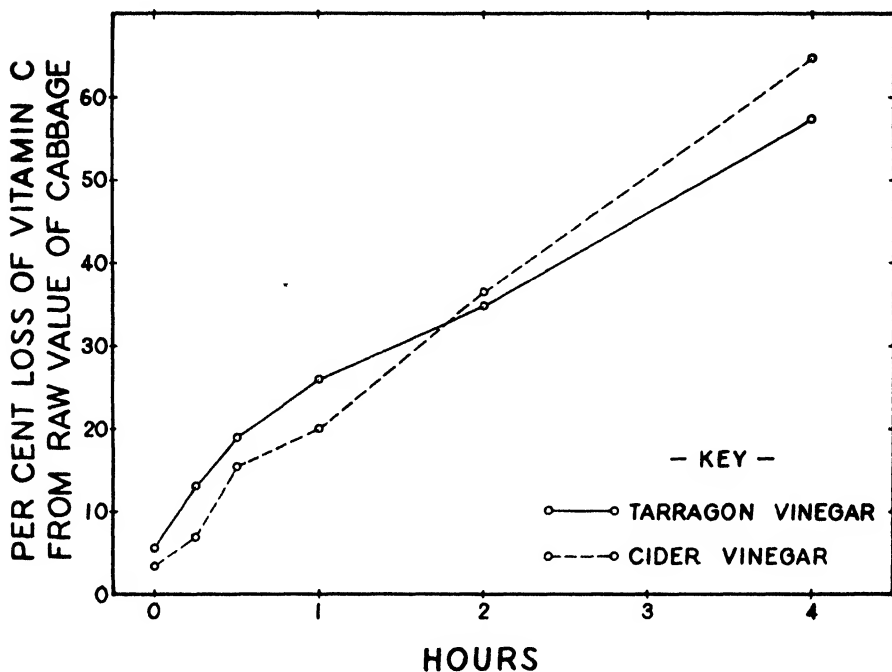
FIG. 1. Rate of loss of vitamin C in the cabbage portion of salads (white enameled bowls and metal-tined kitchen fork used) made with French dressing containing cider or tarragon vinegar of five per cent acidity.

RESULTS

The results of the experiments on cabbage are summarized (Fig. 1 and Tables 2, 3, 4, and 5).

In cabbage salads made with French dressing containing cider or tarragon vinegar of five per cent acidity (Fig. 1) the cabbage lost vitamin C quite rapidly after the addition of dressing containing either kind of vinegar.[4] The immediate loss was somewhat more rapid with the tarragon vinegar dressing but was higher after four hours with the cider vinegar dressing. At the end of two hours the loss was approximately 35 per cent with both kinds of dressing. In this same time interval the loss in the same amount of plain shredded cabbage in the same kind of bowl was only 0.5 per cent.

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[4] Similar results were obtained for kale when it was mixed with French dressing containing cider vinegar of five per cent acidity. See Maine Agr. Expt. Sta. Bull. 438, Report of progress for year ending June 30, 1945.

The effect of kind and acidity of vinegar (or acetic acid), when used in French dressings, on the average rate of loss of vitamin C in cabbage salads is shown (Table 2). After two hours the percentage losses of vitamin C were very similar in salads made with French dressing containing the different kinds of vinegar of the same acidity. Also the losses with acetic acid were similar to those with vinegars of the same acidity. In two hours the average percentage loss of vitamin C in salads made with French dressing containing five per cent vinegars or acetic acid was 34.3 per cent. In those containing three per cent vinegar or acetic acid the loss was 21.5 per cent, and in salads made with a control mixture containing distilled water instead of the vinegar of the French dressing the loss was only 0.8 per cent. These differences associated with relative acidity were shown to be highly significant statistically. Enameled bowls and a kitchen fork were used in this series.

TABLE 2

Effect of Kind and Acidity of Vinegar (or Acetic Acid) When Used in French Dressings on Average Rate of Loss of Vitamin C in Cabbage Salads[1]

Kind and acidity of vinegar	Number of tests	Raw cabbage, immediate test	Cabbage in salad, immediate test	Loss from raw value	Cabbage in salad, 2-hr. test	Loss from raw value
		Vitamin C	Vitamin C[2]		Vitamin C[2]	
		mg./ 100 gm.	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*pct.*
Cider, 5%	2	50.31	48.64	3.4	33.78	32.7
Distilled white, 5%	2	33.39	32.24	3.1	20.97	37.9
Malt, 5%	2	39.89	39.05	2.1	27.68	30.8
Tarragon, 5%	2	33.54	31.66	5.5	20.72	38.3
Acetic acid, 5%	2	46.69	46.03	1.5	32.04	31.4
Average	....	40.76	39.52	3.1	27.04	34.3
Cider, 3%	2	40.73	38.09	6.4	32.53	20.3
Distilled white, 3%	2	34.88	33.72	3.1	27.44	21.9
Malt, 3%	2	31.37	31.09	1.2	24.75	21.4
Tarragon, 3%	2	33.54	31.38	6.4	25.70	23.3
Acetic acid, 3%	2	40.86	40.36	1.5	32.50	20.7
Average	....	36.28	34.93	3.7	28.58	21.5
Distilled water (instead of vinegar in dressing)	2	36.15	34.83	3.7	35.88	0.8

[1] White enameled bowls and metal-tined kitchen fork used in this series. [2] Results calculated on basis of original weight of cabbage in individual salads (50 gm.); 10 gm. dressing in each.

The effects of seven different kinds of plain vinegars and acetic acid (all diluted to three per cent acidity)[5] on average vitamin C losses in cabbage salads are shown (Table 3). In these experiments 3.4 gm. of diluted vinegar or three per cent acetic acid were used with 50 gm. of cabbage. This is the amount of vinegar contained in 10 gm. of French dressing. A statistical study of the results obtained in the two-hour tests indicates that, while there was some variability in the results with the different vinegars, these differences were not significant. Also the results with the three per

[5] All of the vinegars in this series were diluted to three per cent acidity because some were below five per cent as purchased.

cent acetic acid were not significantly different from those with the vinegars. Glass bowls and a silver fork were used in this series.

TABLE 3

Effect of Different Kinds of Plain Vinegars (and Acetic Acid) Diluted to Three Per Cent Acidity, on Average Vitamin C Losses in Cabbage Salads[1]

Kind of vinegar	Number of tests	Raw cabbage, immediate test Vitamin C	Cabbage in salad, immediate test Vitamin C[2]	Loss from raw value	Cabbage in salad, 2-hr. test Vitamin C[2]	Loss from raw value
		mg./ 100 gm.	mg./ 100 gm.	pct.	mg./ 100 gm.	pct.
Cider	2	23.28	22.50	3.5	15.39	33.9
Distilled white	2	23.28	21.83	6.3	15.13	35.1
Malt	2	23.93	22.82	4.8	17.32	28.0
Tarragon	2	27.92	26.66	4.5	17.60	37.0
Herb salad	2	27.90	25.40	8.5	19.47	30.3
Red wine	2	20.76	19.39	6.6	13.71	34.0
Sauterne	2	20.76	18.69	9.7	12.68	39.0
Acetic acid	2	42.44	39.96	6.2	26.94	37.1
Average	....	26.28	24.66	6.3	17.28	34.3

[1] Glass bowls and silver fork used in this series. Vinegar or acid diluted with glass distilled water. [2] Results calculated on basis of original weight of cabbage in individual salads (50 gm.). Each salad contained 3.4 gm. vinegar (the amount in 10 gm. French dressing).

Effects of utensils used in making cabbage salads containing plain vinegars (or acetic acid) on the average rate of loss of vitamin C are indicated (Table 4). After two hours the percentage losses of vitamin C were 12.7 per cent higher in the salads made with plain five per cent cider vinegar or acetic acid when enameled bowls and a kitchen fork were used instead of glass bowls and a silver fork. When the salads were made with three per cent vinegar the differences owing to the utensils averaged only 4.5 per cent.

Effects on average losses of vitamin C of the addition of seasonings to cider vinegar or acetic acid used in cabbage salads are presented (Tables 2 and 4). A comparison of the results indicates that the cabbage in salads made with plain five per cent cider vinegar lost 34.6 per cent more vitamin C than that in salads made with French dressing containing the same amount of the same kind of vinegar (enameled bowls and kitchen fork used). Similar comparative results were secured with five per cent acetic acid and French dressing containing the same strength of acetic acid. It therefore appears that the oxidizing effect of the vinegar or acid is retarded by other ingredients in the French dressing.

In an attempt to determine whether it was the oil or the seasonings which had the retarding effect on oxidation a series of tests was carried out using plain vinegar (five per cent acidity) or five per cent acetic acid plus the seasonings used in the French dressing. The results of these experiments are given (Table 5). Comparison is made with results previously obtained with plain cider vinegar or acetic acid and with French dressing containing cider vinegar or acetic acid. It is apparent that vitamin C losses were much lower in the salads made with the vinegar or acetic

TABLE 4

Effect of Utensils Used in Making Cabbage Salads Containing Plain Vinegar (or Acetic Acid)[1] on Average Rate of Loss of Vitamin C

Acidity of vinegar or acetic acid	Type of bowl and fork	Number of tests	Raw cabbage, immediate test	Cabbage in salad, immediate test	Loss from raw value	Cabbage in salad, 2-hr. test	Loss from raw value
			Vitamin C	Vitamin C[2]		Vitamin C[2]	
			mg./ 100 gm.	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*pct.*
Cider, 5%..............	Enameled bowl and kitchen fork	2	36.31	34.28	6.0	11.76	67.3
Acetic acid, 5%.......	Enameled bowl and kitchen fork	2	39.10	36.04	8.2	12.56	67.4
Average..............	....	....	37.71	35.16	7.1	12.16	67.4
Cider, 5%..............	Glass bowl and silver fork	3	31.42	29.61	5.3	14.86	53.1
Acetic acid, 5%.......	Glass bowl and silver fork	3	33.15	31.42	5.2	14.10	56.3
Average..............	....	....	32.29	30.52	5.3	14.48	54.7
Cider, 3%..............	Enameled bowl and kitchen fork	2	36.31	32.64	10.3	22.45	38.4
Cider, 3%..............	Glass bowl and silver fork	2	23.28	22.50	3.5	15.39	33.9

[1] Each salad contained 3.4 gm. of vinegar or acid (the amount in 10 gm. French dressing).
[2] Results calculated on basis of original weight of cabbage.

TABLE 5

Effect on Average Losses of Vitamin C of Addition of Seasonings to Cider Vinegar or Acetic Acid Used in Cabbage Salads

Kind of dressing	Number of tests	Raw cabbage, immediate test	Cabbage in salad, immediate test	Loss from raw value	Cabbage in salad, 2-hr. test	Loss from raw value
		Vitamin C	Vitamin C[1]		Vitamin C[1]	
		mg./ 100 gm.	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*pct.*
Cider vinegar, 5%[2]................................	3	31.42	29.61	5.3	14.86	53.1
Acetic acid, 5%[2].................................	3	33.15	31.42	5.2	14.10	56.3
Cider vinegar, 5%, plus seasonings[2].....	2	46.13	44.46	4.0	34.16	27.1
Acetic acid, 5%, plus seasonings[2]........	2	38.43	37.58	2.2	28.83	25.1
French dressing[3] with 5% cider vinegar........................	2	50.31	48.80	3.0	31.31	25.8
French dressing[3] with 5% acetic acid.............................	2	46.69	46.22	1.0	34.46	26.2

[1] Results calculated on basis of original weight of cabbage in individual salads. Each salad contained 10 gm. French dressing or the equivalent amount of vinegar or vinegar plus seasonings. [2] Glass bowls and silver fork used. [3] Since enameled bowls and kitchen fork were used for the salads containing French dressing, the values given have been corrected to make them comparable to those given for the vinegar and acetic acid and the vinegar and acetic acid plus seasonings.

acid containing the seasonings than in those containing vinegar or acid alone. The losses in salads made with French dressing were almost identical with those in salads made with equivalent amounts of the vinegar or acid plus seasonings. (Analysis of the freshly mixed seasonings showed that they contained only a trace of vitamin C.) Therefore it appears that the lower losses of vitamin C which occur in cabbage salads made with French dressing (as compared with those made with plain vinegar) are chiefly due to the seasonings in the dressing rather than the oil.

DISCUSSION

According to Wachholder (1942) the vitamin C in cabbage can be oxidized by a heat-labile ascorbic acid oxidase and by heat-stable metal containing catalysts. Bach (1945) states that at a pH of 6.5 an aqueous vapor distillate of cabbage is inhibitory to the catalytic effect (on vitamin C oxidation) of $CuSO_4 \cdot 5H_2O$ in a concentration of 5.7×10^{-5} M but not at a concentration of 5.7×10^{-4} M. She explains this inhibitory action by the fact that the distillate contains sulphur compounds which probably combine with copper to form sulfides.

In the experiments reported in the present paper the damage to the cells of the cabbage during shredding with a stainless-steel knife was apparently not great enough to release the ascorbic acid oxidase. Therefore the protective substances present in the cabbage were adequate to protect the vitamin C in the plain shredded cabbage and in the cabbage mixed with the French dressing containing distilled water instead of vinegar (Table 2). However, when vinegar or acetic acid was added to the cabbage, either plain or in French dressing, metallic ions were probably liberated which catalyzed the oxidation. The source of part of these ions was probably in the traces of metallic salts which were present as contamination. Also the increase in acidity was apparently sufficient to free other metallic ions which had previously been combined with the protective substances. This explanation appears logical, since the loss of vitamin C in the cabbage was proportional to the titratable acidity of the vinegar or acetic acid.

The theoretical pH of five per cent acetic acid is 2.4 and of three per cent, is 2.5. According to Tauber, Kleiner, and Mishkind (1935) inactivation occurs in purified squash oxidase at pH 2. Therefore it does not seem probable that the ascorbic acid oxidase in the cabbage was activated by the vinegar or acetic acid used in the cabbage salads.

The increased oxidation of vitamin C which occurred when the enameled bowls and kitchen fork were used in the presence of vinegar or acetic acid can be explained as being due to the effect of additional metallic ions from the bowls and fork.

Strohecker, Busse, and Weinreich (1941) found that of a number of metals which they tested silver and copper had the strongest action in catalyzing vitamin C oxidation. Therefore, it seemed possible that the silver fork, used in some of the experiments reported in the present paper, might have provided silver ions which were partly responsible for the vitamin C oxidation. However, in a series of experiments in which a comparison was made of the effect of mixing cabbage salads containing French

, ressing with a recently polished silver fork or with one of a transparent
lastic material, no significant difference was shown. In these experiments
+he salads were mixed in glass bowls and held at room temperature for
one- and two-hour intervals before testing.

The question arises as to why there was a significant difference between
the results with vinegars and those with acetic acid in the experiments with
pure ascorbic acid and in those with extract of green pepper but none in
the cabbage experiments. The answer to this question may be given by
pointing out certain differences in the conditions existing in the different
experiments. In the experiment with the solution of pure ascorbic acid
(Table 1) the only metallic ions (such as iron or copper) introduced were
those in the vinegar itself; therefore the oxidation was more rapid in the
mixtures containing vinegar. In the experiment with the extract of green
pepper in eight per cent acetic acid, Clayton (1940), only a small amount
of the pepper extract (probably containing only traces of metallic ions)
was used, so the catalytic effect of the metallic ions in the different vinegars
(but not in the acetic acid) caused differences in the rate of vitamin C
oxidation in the pepper extract.

In the cabbage experiments the amount of cabbage used in proportion
to acetic acid or vinegar was large. Since the cabbage itself probably con-
tained traces of metals which could be ionized by acid and since there was
also some contamination by traces of metals in the utensils used, a differ-
ence between the effect of acetic acid and vinegar was not apparent. It is
also possible that if shorter time intervals had been used in the cabbage
experiments, differences could have been shown between the effects of the
different kinds of vinegar. However, the acidity of the vinegar or acetic
acid appears to be the most important factor responsible for loss of vitamin
C in the cabbage salads.

The protective effect of the seasonings cannot be definitely explained.
They may have contained reducing substances which did not affect the
indicator in the vitamin C titrations. Also, they probably decreased the
acidity of the vinegar and acetic acid to some extent.

As a practical application of the above findings it is suggested that in
order to obtain the maximum amount of vitamin C from cabbage salads
containing vinegar or French dressing the vinegar or dressing should be
added to the cabbage just before eating. The same suggestion applies to
salads made with other greens. Contact of the salads with metals, espe-
cially iron and copper, should be avoided as much as possible.

SUMMARY

Vitamin C losses were studied in individual cabbage salads made with
different kinds of vinegar (or acetic acid) and with French dressings
containing vinegars (or acetic acid). The losses in salads made with
French dressing were lower than in those containing the same amount of
plain vinegar. After the salads had stood two hours at room temperature
the loss of the vitamin was proportional to the titratable acidity of the
vinegar (or acetic acid). This was apparently due to the liberation of
metallic ions by the acid.

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NUTRITIVE VALUE OF FISH FROM MICHIGAN WATERS

II. THIAMIN OF LAKE HERRING, CARP, COMMON SUCKER, BURBOT, AND SMELT [1]

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The nicotinic acid content of several species of fish from Michigan waters of the Great Lakes was reported in a previous publication by Klocke, Porter, Tack, Leffler, Henry, and Nitchals (1946). In the present investigation the thiamin content of lake herring [*Leucichthys artedi*), common suckers (*Catostomus c. commersonii*), carp (*Cyprinus carpio*), burbot (*Lota lota maculosa*), and smelt (*Osmerus modax* [Mitchell]) have been recorded, including the effect of freezing, refrigeration, holding, baking, frying, and possible seasonal and environmental variations.

EXPERIMENTAL PROCEDURE

The lake herring used in the study were caught by Michigan fishermen in the vicinity of Escanaba on Green Bay or East Tawas and Bay Port on Saginaw Bay. The carp were from Monroe on Lake Erie and Saugatuck on Lake Michigan, the common suckers from Cheboygan on Lake Huron, the burbot from Escanaba on Green Bay, and the smelts from Saginaw Bay. The commercial shipping procedure of packing the fish in cracked ice in wooden boxes was followed. The fish arrived one or two days after being caught and were immediately dressed and filleted. A commercial cold-storage plant froze the individually wrapped fillets. The fish which were to be analyzed fresh were kept in the freezing compartment of a seven-cubic-foot refrigerator.

Series of thiamin assays were made on raw, baked, fried, frozen, and refrigerated fish with enzyme digestion followed by oxidation of thiamin to thiochrome. The freezing and baking methods are given in detail in the previous paper by Klocke *et al.* (1946). One-half fillet of fish for frying was placed, flesh side down, in a hot, cast-iron frying pan containing five grams of melted hydrogenated fat. After three to four minutes the fillet was turned and then fried slowly for an additional three to four minutes. The entire contents of the frying pan were analyzed. Herring used for a refrigeration holding test were wrapped individually in parchment paper. One half of each fillet was analyzed immediately and the other half was held for 48, 120, and 137 hours at 4°C.(39°F.).

For extraction of thiamin, one-half fillet of herring or cross sections of the larger species or several smelts were weighed and macerated for

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five to seven minutes in a Waring blendor with five to seven times the tissue weight in water. Duplicate or triplicate samples weighing approximately 40 grams were introduced into 125-ml. Erlenmeyer flasks. To each was added 10 ml. of sodium acetate-acetic acid buffer (pH 4.5) containing 0.2 gm. of the enzyme polidase-S.[2] After enzyme digestion had proceeded for 16 to 18 hours at 45°C.(113°F.), the digest was heated to boiling, cooled, and filtered through Whatman No. 12 filter paper.

Five milliliters of the digested filtrate were pipetted into glass-stoppered cylinders. In rapid succession three drops of one-per cent potassium ferricyanide, three ml. of 15-per cent sodium hydroxide, and 30 ml. of distilled isobutanol were added. The contents of the cylinders were shaken for one and one-half minutes, poured into separatory funnels, and the layers allowed to separate. The alkali layer was discarded and the alcohol layer was poured into a clean, dry, 50-ml. centrifuge tube. Four grams of anhydrous sodium sulfate were added to each tube and the tubes were centrifuged for approximately two minutes or until the solution was crystal clear. The fluorescence produced was measured with a Lumatron, using a solution of crystalline thiamin oxidized to thiochrome as a primary standard and quinine sulfate as a secondary standard. Blank readings were made on every extract by omitting the potassium ferricyanide.

Two weighed portions of the macerated tissue were dried overnight at 95 to 100°C.(203 to 212°F.) to determine total solids, brought to room temperature in a desiccator, and weighed. The percentages of solids were calculated.

Analysis and calculations were made on the edible portion of the fish. The reported values for fried and baked fish were calculated by multiplying the micrograms per gram of cooked tissue by the cooked weight and dividing by the raw weight. The significance, Snedecor (1940), of the differences in the results of method variations and processing were determined by the "t" test.

RESULTS

A preliminary comparison of methods of extraction and hydrolysis of thiamin from fish tissue was made using suggested enzymes and incubation periods (Table 1). The results of Series I indicate that a combination of the enzymes takadiastase and papain gave less than 10 per cent lower values than polidase-S alone. In Series II the results of incubation with polidase-S for two and one-half or 16 to 18 hours were comparable. Mild acid hydrolysis, Series III, in which the samples were refluxed on a steam bath for 60 minutes followed by enzyme digestion with polidase-S, gave results corresponding to direct digestion with polidase-S. Enzyme digestion with polidase-S for 16 to 18 hours at 45°C. was considered adequate and practical for the tissues being analyzed in this laboratory. Base-exchange tubes have been suggested for the removal of fluorescing substances occurring naturally in tissues. However, the adsorption step in the method of Conner and Straub (1941) was found unnecessary because the thiamin values obtained by its use were not significantly higher (Series IV).

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[2] Recommended by Clausen and Brown (1943) and obtained from Schwartz Laboratory.

The thiamin contents of lake herring, carp, common suckers, burbot, and smelts are presented (Table 2). The average thiamin values for 11 groups of lake herring ranged from 0.27 to 1.24 µg. of thiamin per gram of moist tissue with an average of 0.87 µg. per gm. for a total of 140 fish. Burbot had an average value of 3.78 µg. of thiamin per gm. of moist tissue. The values for herring were within the range of 0.3 to 2.4 µg. per gm. reported for salt-water fish by Munsell (1940), Lovern (1943), Leong (1940), and Holmes (1945), and those for the burbot were slightly higher. Judging from the present results, common suckers, carp, and smelts contain insignificant amounts of thiamin. The absence of thiamin in raw carp,

TABLE 1

A Comparison of Methods of Extraction and Hydrolysis of Thiamin From Lake Herring Using Suggested Enzymes and Incubation Periods

Sample No.	Series I Enzyme digestion, 16-18 hr. pH 4.5, at 45°C.		Series II Digestion with polidase, pH 4.5, at 45°C.		Series III Digestion with polidase for 16-18 hr., pH 4.5, at 45°C.		Series IV Digestion with polidase, 16-18 hr., pH 4.5, at 45°C.	
	Takadiastase and papain	Polidase	2½ hr.	16-18 hr.	Refluxed 60 min. on steam bath	No refluxing	With absorption on decalso	Without absorption
	µg./gm.	*µg./gm.*	*µg./gm.*	*µg./gm.*	*µg./gm.*	*µg./gm.*	*µg./gm.*	*µg./gm.*
1	0.45	0.49	0.51	0.49	0.48	0.49	......	......
2	1.10	1.22	......	......	......	......	......	......
3	1.08	1.11	......	......	......	......	......	......
4	0.95	0.97	......	......	......	......	......	......
5	......	......	......	......	1.47	1.58	1.47	1.58
6	......	......	......	......	1.38	1.40	1.34	1.40
7	......	......	......	......	......	......	1.27	1.27
8	......	......	......	......	......	......	1.58	1.62
9	......	......	......	......	......	......	0.87	0.91
10	......	......	......	......	......	......	0.62	0.68

smelts, and ocean herring has been investigated by Spitzer, Coombes, Elvehjem, and Wisnicky (1941); Deutsch and Ott (1942); Sealock, Livermore, and Evans (1943); Owen and Ferrebee (1943); and Krampitz and Woolley (1944). These investigators have found a substance which causes an enzymatic degradation of the thiamin molecule. The viscera contain the highest concentration of the active component. Foxes fed carp viscera developed a thiamin deficiency but those fed carp fillets were normal. Since Smith and Proutt (1944) reported that cats fed exclusively on diets of raw carp or raw ocean herring developed a characteristic thiamin deficiency but those fed perch, catfish, or butterfish did not, the thiamin-destroying component probably is present only in certain species of fish. Woolley· (1941) and Owen and Ferrebee (1943) have found that the factor was destroyed by cooking or autoclaving, drying, and peptic digestion.

Since the diet of the animal influences to some extent the nutritive value of the tissue, Miller, Pence, Dutcher, Ziezler, and McCarty (1943), lake herring were analyzed during the two yearly runs, in May and June

and November and December, and also in January and February, when they are less abundant. Herring in the spring run of 1944 averaged 1.06 μg. of thiamin per gm., and in 1945 when the skin was included in the analysis, they averaged 0.73 μg. per gm. The average of 76 herring analyzed during the two spring runs was 0.89 μg. per gm. of fresh tissue. Thirty-two herring caught and analyzed during the fall spawning season averaged 0.58 μg. per gm. and the 32 fish assayed in January and February averaged 1.12 μg. per gm. A total of 63 fish from Saginaw Bay contained an average of 0.86 μg. per gm., and a total of 61 fish from Green Bay had an average value of 0.87 μg. per gm. Seasonal and environmental conditions and individual variations are probably sufficient to explain the range in values for the herring assayed in this study.

The data from the seven lots of herring analyzed fresh and after two and one-half to 11 months frozen storage (Table 2) indicate that the loss of thiamin is either slight or insignificant. In six lots the loss was insignificant and one was slightly significant ("t" = 3.4 with 23 D.F.). In burbot there is an apparent increase in the thiamin content; however, the per cent solids increased from 17.9 to 25.4, which explains the apparent increase in thiamin content.

A significant loss ("t" = 5.03 with 16 D.F. and 5.06 with 40 D.F.) of thiamin occurred when two batches of lake herring were fried (Table 3). There was an average of 88 and 84 per cent retention of the vitamin. Lane, Johnson, and Williams (1942) reported a 60-per cent retention of thiamin for fried flounder but did not give cooking methods.

With the baking methods used in this study, lake herring lost insignificant amounts of thiamin (Table 4). According to Lane, Johnson, and Williams (1942), baked halibut retained 55 per cent of the thiamin. Holmes (1945) found 40 to 90 per cent retention in five species of sea fish cooked by institutional methods when the fish were baked 30 to 40 minutes at 232°C.(450°F.). Since McIntire, Schweigert, Henderson, and Elvehjem (1943) state that method of cooking and size of cut have an effect on the per cent retention of vitamins in meat, the fish in this experiment which were baked for 15 minutes at 177°C.(350°F.) should have retained more thiamin than those baked for a longer period of time at a higher temperature. Cover, McLaren, and Pearson (1944) reported a higher retention of thiamin in ribs of beef baked rare than in those baked well done.

The data from two groups of herring fillets held at 4°C.(39°F.) indicate that some loss of thiamin occurs during refrigeration (Table 5). In Series I the loss was insignificant at the end of 48 hours, but in Series II the loss was significant ("t" = 3.04 with 22 D.F.). Holding periods of 120 and 137 hours showed significant losses ("t" = 3.45 and 3.49 with 22 D.F.). In the second series, part of the fish were frozen dressed, stored for about four weeks, and shipped while frozen. The fish were not allowed to thaw and were not filleted until the analysis was made five weeks after harvesting. When this group is compared with those shipped on ice and analyzed immediately, there is a slightly significant loss ("t" = 2.52 with 28 D.F.). Further tests should be made on the effects of similar types of refrigeration.

TABLE 2

Thiamin Content of Fresh and Frozen Lake Herring, Carp,
Common Suckers, Burbot, and Smelts

Species	Season caught	Period of frozen storage	Number of samples	Thiamin in raw tissue		Solids	
				Mean	S.D.[1]	Mean	S.D.
Lake herring		mo.		µg./gm.	µg./gm.	pct.	pct.
Saginaw Bay	Nov. 1943	14	4	0.92	....	21.7	......
	June 1944	0	20	1.08	0.19	20.6	1.5
		2½	17	1.00	0.14	19.8	1.8
		7	20	1.26	0.25	20.5	1.9
		11	21[2]	0.94	0.14	21.2	2.7
	Nov. 1944	0	5	0.80	......	20.1	......
		4	12	0.60	0.20	18.0	1.9
	Dec. 1944	0	10	0.27	0.16	18.8	1.7
	June 1945	0	18[2]	1.04	0 15	21.6	2.9
		0	10[2]	0.70	0.21	22.5	2.7
Green Bay	June 1944	0	18	0.82	0.13	21.7	2.1
		2½	15	0.60	0.13	22.2	1.7
	Nov. 1944	0	7	0.43	......	19.1	......
		5	12	0.36	0.12	19.6	2.4
	Dec. 1944	0	10	0.86	0.12	18.4	1.5
	Jan. 1945	0	16	1.24	0.15	18.9	1.7
		6	9[2]	1 00	0.12	18.9	1.0
	Feb. 1945	0	16	1.00	0.33	17.2	1.7
	June 1945	0	10[2]	0.59	0.09	20.4	1.8
Common suckers	March 1944	0	2	0.0	......	......	......
	Dec. 1944	0	4	0.04	... ..	21.1	......
Carp	May 1944	3	2	0.05	......	21.3	......
	Oct. 1944	3	2	0.0	......	......	......
Burbot	Nov. 1944	0	6	3.78	......	17.9	......
		3½	6	4.44	......	25.4	......
	March 1945	½	5	4.33	0.81	15.6	......
Smelts	May 1945	0	17[2]	0 05	0.03	19.2	1.4

[1] S D. means standard deviation. [2] Skins included in ana

TABLE 3

Thiamin Content of Raw and Fried Lake Herring

Source	Number of samples	Thiamin in tissue [1]				Solids			
		Raw		Fried [2]		Raw		Fried	
		Mean	S.D.[3]	Mean	S.D.	Mean	S D.	Mean	S.D.
Saginaw Bay		µg./gm.	µg./gm.	µg./gm.	µg./gm.	pct.	pct.	pct.	pct.
Fresh..........	3	1.04	......	0.98	......	23.0	......	40.8	......
Green Bay									
Frozen.......	9	1.00	0.12	0.88	0.12	18.9	1.0	39.9	6.2
Saginaw Bay									
Frozen.......	21	0.94 [4]	0.14	0.79	0.13	21.2	2.7	52.1	8.0

[1] Skins included in analysis. [2] Reported value on fried portion obtained by multiplying µg. per gm. fried weight by fried weight and dividing by weight before cooking. [3] S.D. means standard deviation.

After analyzing the average American diet, Lane, Johnson, and Williams (1942) concluded that meats supply approximately one-fourth of the total thiamin in the diet. Since the thiamin content of lake herring and burbot is comparable to that of lean meats, with the exception of pork, these fish and undoubtedly others which have not been analyzed can make a valuable contribution to the American diet. Better shipping and marketing practices would do much to increase the consumption of this highly nutritious food.

TABLE 4

Thiamin Content of Raw and Baked Lake Herring

Source	Number of samples	Thiamin content				Solids			
		Raw		Baked [1]		Raw		Baked	
		Mean	S.D.[2]	Mean	S.D.	Mean	S.D.	Mean	S.D.
		µg./gm.	*µg./gm.*	*µg./gm.*	*µg./gm.*	*pct.*	*pct.*	*pct.*	*pct*
Green Bay Fresh..........	8	0.86	0.12	0.85	0.33	18.4	1.5	20.5	2.6
Saginaw Bay Frozen..........	16	1.26	0.25	1.25	0.22	20.5	1.9	24.4	2.5

[1] Sample weighed before baking and entire sample plus drippings used for analysis   [2] S.D. means standard deviation.

TABLE 5

Influence of Refrigeration on Thiamin Content of Lake Herring

Series	Period of storage [1]	Number of samples	Thiamin in raw tissue		Solids	
			Mean	S.D.[2]	Mean	S.D.
	hr.		*µg./gm.*	*µg./gm.*	*pct.*	*pct.*
I	0	16	1.24	0.15	18.9	1.7
	48	8	1.27	0.29	19.4	1.6
	120	8	1.16	0.10	......	......
II	0	16	1.00	0.33	17.2	1.7
	48	8	0.69	0.11	17.2	
	137	8	0.53	0.14	18.7	1.8
	5(wk.)[3]	14	0.80	0.16	19.3	0.9

[1] Filleted, individually wrapped in parchment paper, stored at 4°C.(39°F.).   [2] S.D. means standard deviation.   [3] Frozen immediately, dressed, held frozen until analyzed, then filleted.

SUMMARY AND CONCLUSION

The influence of season of the year, environment, freezing, refrigeration holding, baking, and frying on the thiamin content of five species of fish from Michigan waters, including carp, lake herring, common suckers, burbot, and smelts, has been reported. In this study thiamin was determined by applying the thiochrome method to enzyme-digested samples.

The lake herring from Saginaw Bay analyzed during three seasons averaged 0.86 µg. of thiamin per gram of fresh tissue, and during four seasons those from Green Bay averaged 0.87 µg. per gm. Herring assayed during the spring run on two successive years averaged 0.89 µg. per gm.; during one fall spawning season, 0.58 µg. per gm.; and during January and February, 1.12 µg. per gm. Individual variation and seasonal and environmental conditions can probably account for the significant differences found in the herring studied.

Burbot averaged 3.78 μg. of thiamin per gm. of tissue. No thiamin was found in the carp, common suckers, and smelts which were analyzed.

Freezing and subsequent storage for two and one-half to 11 months caused slight to insignificant losses of thiamin in seven lots of herring. Under the conditions of the experiment, baking losses of thiamin in two series of observations of herring were insignificant. Two lots of fried herring retained an average of 84 and 88 per cent of the original thiamin. The data from two groups of lake herring indicate that some loss of thiamin may occur after refrigeration for 48, 120, and 137 hours at 4°C.(39°F.).

Lake herring and burbot can make a valuable contribution to the thiamin content of the American diet.

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DIRECT COLORIMETRIC METHOD AND ITS APPLICATION TO DETERMINATION OF ENZYMATIC DISCOLORATION IN WHITE POTATOES [1]

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In the production of dehydrated potatoes one of the principal concerns is the avoidance of various types of discolorations which may impair the quality of the finished product.

Such discolorations are both enzymatic and nonenzymatic in nature. They include the so-called "scorch" or heat damage, a reddish-brown discoloration generally arising in the center of the cube or strip, which is associated with the presence of abnormal quantities of reducing sugars; and the graying discoloration, Nelson and Dawson (1944), which is presumably attributable to the action of potato oxidases and molecular oxygen on substrates contained within the potato cell (tyrosine, dihydroxyphenylalanine, etc.) leading to the formation of various pinkish or reddish intermediaries and finally to the formation of black-potato melanin. The reaction does not occur within the intact cell but is initiated by peeling, cutting, freezing, bacterial infection, or other damage.

In the dehydration process the cut dice or slices are blanched by boiling water or steam to destroy enzymatic activity prior to their dehydration in the air tunnel. In "satisfactory" potatoes, pinking is slow so that no significant discoloration occurs before the enzymatic discoloration is halted in the blanch. Certain potatoes, however, exhibit abnormal pinking resulting in appreciable discoloration in the finished product, Ross, Hilborn, and Jenness (1945). Blanching greatly improves the quality of dehydrated vegetables, according to Cruess (1943). Excessive blanching, however, is destructive of quality, Campbell, Lineweaver, and Morris (1945), so that a precise control of the blanching procedure is of considerable importance.

To test the completeness of blanching, various methods have been employed, Proctor (1942), for testing residual enzymatic activity involving the application of indicators susceptible to enzymatic oxidation on the cut surface of the potato slice or die (e.g., guaiacol benzidine or gum guaiac for peroxidase; catechol and p-cresol for oxidase).

A mere examination of the surface, however, may not yield a true picture of the discoloration tendency of the entire die because the inner parts of the dice may differ from the cut surfaces.

In the following method the enzymatic discoloration tendency of potatoes is determined by the degree of coloration formed by the interaction

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[1] The subject matter of this paper was undertaken in co-operation with the Quartermaster Corps Committee on Food Research.

of potato oxidase and potato substrate in macerated potato tissue under standard conditions without the addition of external indicator.

EXPERIMENTAL PROCEDURE

In general, the method is based upon the colorimetric measurement of the depth of color of potato juices when obtained in a uniform manner. Such measurement can then be related to any set of conditions to which the potatoes may have been subjected prior to preparing the juice from them. In the preparation of a juice suitable for colorimetric tests several precautions have to be taken to insure reproducibility. It is important to maintain the time intervals between the steps of the procedure constant since the extent of discoloration of the juice depends on the length of exposure to air. In order to be truly representative of the enzymatic activity of the entire potato, the material has to be finely macerated. The juice should be brilliantly clear.

Potatoes were washed, peeled under running tap water with a plastic knife, and all blemishes removed. Whenever several potatoes were used in one experiment, they were covered with distilled water [20°C.(68°F.)] immediately after peeling so as to avoid discoloration of the peeled surface.

The peeled potato was put through a metal dicer longitudinally and the resulting strips then diced crosswise giving uniform dice of one cubic centimeter. The dice were covered with distilled water (20°C.) until used.

Maceration of 100 gm. of dice was carried out in a Waring blendor (one-quart capacity) with an equal weight of distilled water (20°C.) plus five c.c. of pancreatic extract.[2] Use of the pancreatic enzyme facilitated subsequent filtration and improved the clarity of the juice. Blending was continued for three minutes. (Longer blending time is inadvisable since the mash discolors rapidly during this procedure unless carried out in an atmosphere of nitrogen.) To cut down the foaming during maceration, a few drops of caprylic alcohol, ethyl acetate mixture (1:1), were added.

Samples of the mash were weighed into Erlenmeyer flasks, stoppered, and shaken in a constant temperature water bath at 38°C.(100.4°F.), 114 oscillations per minute, 3.2 cm. horizontal stroke, for one-half hour.

At the end of the shaking period one gram of Filter Cel was added per 200 gm. of mash, and the mixture was then filtered by suction through a Filter Cel cake and Whatman filter paper No. 5 into test tubes containing Nujol, which prevents further contact with the air. The contents of all the tubes of one sample were pooled under Nujol and filtered by gravity through Whatman filter paper No. 5 into Klett tubes containing Nujol. The resulting filtrate was absolutely clear. For any given experiment the period of time between the removal of samples from the shaker and the reading of the colorimeter was kept constant.

A Klett-Summerson colorimeter with 420 mμ filter was employed to determine density of the sample filtrates. Distilled water was used as the control.

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[2] The extract was prepared by grinding 10 grams of dried pancreas with 100 c.c. of glycerol, mixing it with 100 c.c. of water, centrifuging, and filtering by suction through a Filter Cel cake and Whatman filter paper No. 5.

Reproducibility of Results With Samples of a Given Batch of Potatoes: To establish the constancy of extent of coloration on potato juice following the procedure of the direct colorimetric method, five 100-gm. samples of dice from a batch of well-mixed potatoes were employed. Each sample was treated individually, care being taken to maintain comparable time intervals between each step of the procedure for all the samples (Table 1).

TABLE 1

Extent of Coloration in Batch of Mixed Potatoes Determined Colorimetrically

Sample No.	Blend	Shake	Suction filter	Gravity filter	Colorimeter		Deviation	
					Time	Reading	Actual	Per cent
1	10:31	10:35	11:05	11:08	11:10	380	—12	—3.6
2	10:41	10:45	11:15	11:18	11:20	410	18	4.6
3	10:51	10:55	11:25	11:28	11:30	393	1	0.25
4	11:01	11:05	11:35	11:38	11:40	400	8	2.0
5	11:11	11:15	11:45	11:48	11:50	375	—18	—4.6

The data show that application of the direct method described for determination of the coloration capacity of a given potato sample assures results having an accuracy with a possible error of four to five per cent. Subsequent experience with the use of this procedure confirmed the reproducibility of results.

Variation of Discoloration Activity Among Individual Potatoes as Determined Colorimetrically: In the course of this investigation, potatoes of the same variety were found to differ in their tendency to become discolored. Further interest led to determining quantitatively these observed differences. Summarized data from 17 potatoes studied (Table 2) indicate that potatoes of a given batch can vary from one another in terms of their coloration capacity by as much as 100 per cent, but the majority of them fall in a similar range. Further, no correlation is observable between the initial weight of the intact potato and its measured coloration activity.

Effects of Blanching Time on Discoloration of Potato Juice: To investigate the applicability of the direct colorimetric method to determination of length of time necessary for satisfactory blanching in terms of enzymatic discoloration of the potato dice, the following experiment was set up.

Dice were prepared from nine potatoes, well mixed, and 100-gm. samples of the dice mixture were weighed out for each blanching period of 0, 10, 20, 30, 40, 50, and 60 seconds. The dice of each sample were spread in a single layer on a net consisting of a double layer of cheesecloth stretched over a glass ring with detachable glass hooks as handles. The net with the dice was then immersed in a pan of boiling water about nine centimeters deep. At the end of the blanching period the dice were cooled on the net in a pan with running cold tap water for about 30 seconds and then subjected to the treatment outlined in the direct colorimetric procedure. A summary of the results is presented (Fig. 1).

The juice prepared from dice samples blanched for 40 seconds and more does not show any significant color increase upon prolonged standing

at room temperature, whereas the juices from inadequately blanched dice do increase in color density. The direct colorimetric method apparently provides simple quantitative means of determining the extent of decrease through blanching of the enzymatic discoloration capacity of the potato (Fig. 1). Further, this method allows easy determination for any given batch of potatoes of the minimum blanching time required to inactivate the enzymes responsible for such discoloration.

BLANCHING TIME AND POTATO DISCOLORATION.
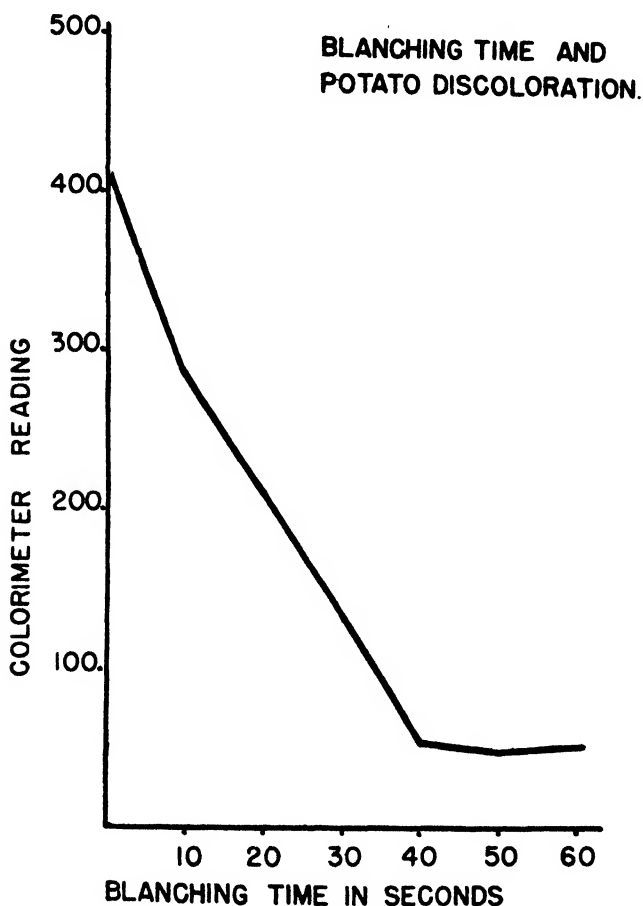
FIG. 1

Having determined the response of a mixture of dice from nine potatoes to length of blanching time, the variation in such response that might exist between individual potatoes from a given batch was next investigated. As already shown (Table 2), individual potatoes do vary with respect to their initial discoloration potentialities. The course of enzyme inactivation with blanching time as well as the possible correlation between the initial discoloration capacity and the time required to destroy it completely were indicated by the following experiment.

Six potatoes weighing 237 to 302 grams were selected. Five 40-gram samples of dice from each potato were subjected to 0, 20, 30, 40, 50, and 60 seconds blanching at 95°C.(203°F.) according to the procedure given above. After the usual treatment the juices were extracted from the mashes and read in the colorimeter.

TABLE 2

Variations in Discoloration of Individual Potatoes

Potato	Weight	Weight of sample	Colorimeter reading
	gm.	*gm.*	
1	127	50	400
2	106	50	425
3	122	50	760
4	167	50	345
5	125	50	340
6	140	50	357
7	148	100	287
8	179	100	410
9	257	100	410
10	137	100	410
11	219	100	530
12	142	100	330
13	134	100	320
14	144	100	385
15	176	100	395
16	172	100	380
17	121	100	345

Examination of the data (Fig. 2) supports the conclusion that while initial coloration capacities of the individual potatoes may vary, the course of the loss of this tendency with blanching is strikingly similar in all cases. Further, on the basis of the data obtained, no correlation between the initial activity of the potato, despite as much as 40 per cent variation, and the extent of its loss in relation to the length of blanching time can be safely drawn. It is evident also that regardless of the initial activity shown by the individual potato, a blanching period of 60 seconds at 95°C. suffices to inactivate completely the juices of all the potatoes used in this experiment.

Ascorbic Acid and Discoloration of Potato Juice: In a preliminary attempt to determine the possible effect that the concentration of ascorbic acid in a particular potato juice might have on the extent of coloration under the direct colorimetric method, samples were prepared of a potato mash to which were added different amounts of ascorbic acid prior to shaking. Colorimetric readings made on the extracted juice after one-half hour of agitation are summarized (Table 3). It is evident that the presence of ascorbic acid, even in an amount greater than 100 per cent increase over what is generally found in potatoes, had no detectable effect upon the extent of discoloration of potato juice.

Effect of Inhibitors on Stabilization of Color of Potato Juice: Since discoloration of unblanched or insufficiently blanched potato juice is a

SODIUM AZIDE INHIBITOR OF POTATO JUICE.

CONTROL

0.001 M

0.005

0.01

0.05

0.1

COLORIMETER READINGS

MINUTES

FIG. 3

BLANCHING OF
INDIVIDUAL POTATOES.

COLORIMETER READINGS.

BLANCHING TIME IN SECONDS
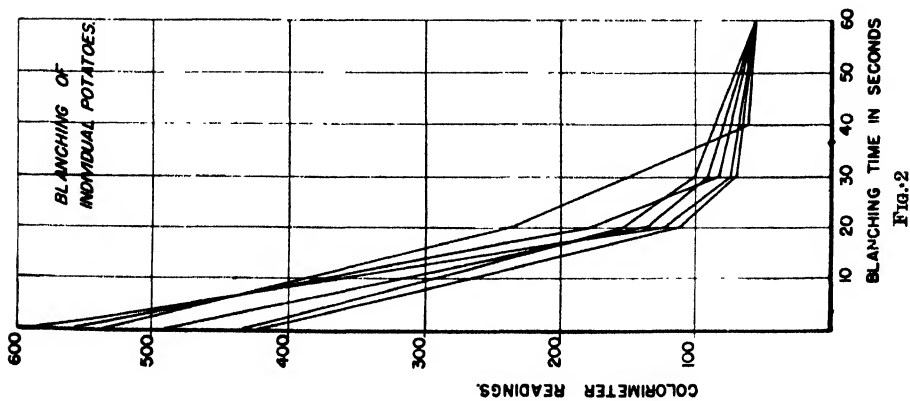
FIG. 2
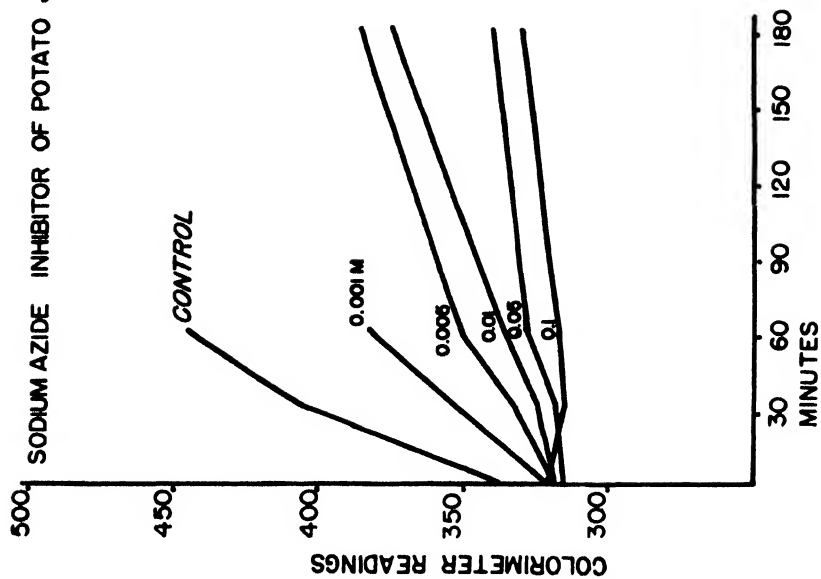
continuous process which necessitates observation of exact time intervals in the readings of the various samples to obtain comparable data, the possibility of arresting color development through the use of suitable inhibitors was investigated. Concentrations of 0.1, 0.01, and 0.001 M of sodium sulfide, hydroxylanine sulfate, sodium hyposulfite, and sodium azide were added to samples of potato juice extracted from a mash prepared in the usual manner from five potatoes. After the initial reading the juices were kept at room temperature in Klett tubes sealed with Nujol and read at the successive intervals given (Fig. 3). Concentrations of 0.1 to 0.05 M sodium azide were satisfactory for maintaining the initial colorimeter values for at least one-half hour without change.

TABLE 3

Effect of Ascorbic Acid Content of Potato Juice on Discoloration

Mash	Plus H$_2$O	Plus 0.2% ascorbic acid	Ascorbic acid per 50 gm. mash	Half-hour colorimetric reading	Deviation from mean
gm.	*c.c.*	*c.c.*	*mg.*		*pct.*
50	5.0	0.0	0.0	342	0.0
50	5.0	0.0	0.0	345	0.7
50	3.75	1.25	2.5	335	2.2
50	2.5	2.5	5.0	348	1.6
50	1.25	3.75	7.5	350	2.2
50	0.0	5.0	10.0	335	2.2

Influence of Blanch-Water Composition on Potato Enzyme Action as Determined by Direct Colorimetric Method: Using the direct colorimetric method, the influence of various agents in the blanching water upon the degree of oxidase action as determined by the amount of coloration of the juice was studied.

The potato dice were washed in cold tap water in an appropriately sized beaker, leaving enough space for floating of the dice during washing, and were agitated by a strong water flow for 90 minutes. By this means, material in the cut cells was removed, and dice of constant coloration were obtained. These were kept in the ice box until the determination.

The test dice were dipped into a flat pan containing four liters of water at 98°C.(208.4°F.) for 20 seconds, then dipped into cold water. Under these conditions the colorimeter reading of the potato was reduced some 40 to 50 per cent as compared with the unblanched control. One hundred grams of the blanched potato were employed for preparation of this juice, this quantity being sufficient to avoid sampling errors. The reading was taken one hour after weighing of the sample.

The blanching agents were added to the blanch water which was maintained at 98°C.(208.4°F.). This temperature rather than boiling was chosen to avoid temperature influences arising from the salt concentration. Tap water was employed for the blanching, but distilled water was used subsequently for the blending.

The results cited were often carried out on different days with different water-blanch control readings as measured on the Klett-Summerson photoelectric colorimeter. To facilitate comparisons the average of water-

blanched potatoes as measured each day was called 100, so that the values
cited for the experimental blanchings represent the coloration as percent-
ages of the control. For the same batch of potato dice the variation rarely
exceeded three or four per cent from the average colorimeter reading.

New Brunswisk, Canada No. 1 potatoes, Long Island, and Maine Green
Mountain potatoes were used in these experiments.

Typical results obtained by this method are shown (Figs. 4, 5, 6, and 7).
The influence of different concentrations of sodium chloride up to 15 per
cent is presented (Fig. 4). The effect of this blanching agent apparently
seems to vary according to the variety of potato.

SODIUM CHLORIDE.

●L.I. GREEN MOUNTAIN POTATOES
○─○NEW BRUNSWICK CANADA No. I
···· COMPOSITE CURVE
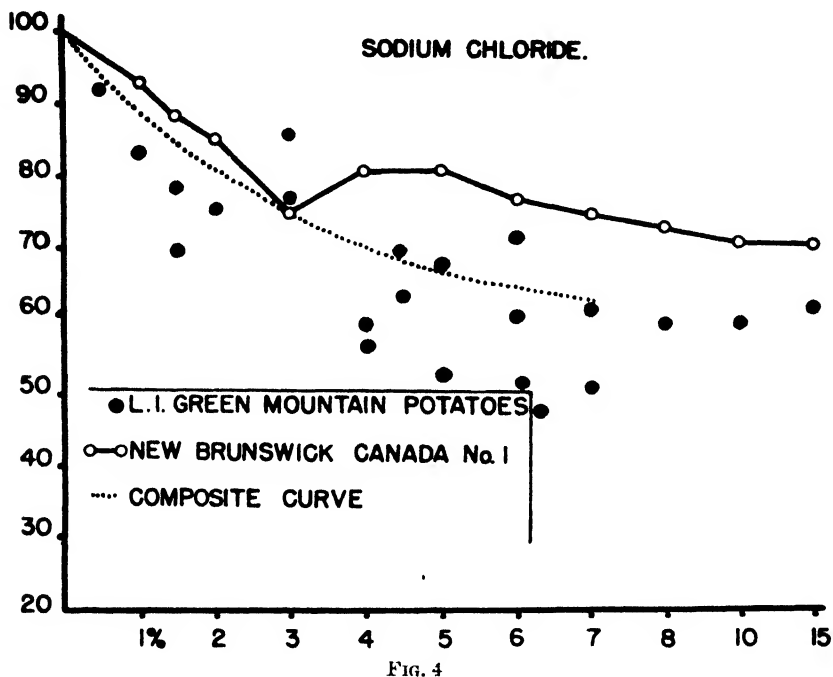
FIG. 4

The influence of three acidic substances, sulphuric and hydrochloric acid
and sodium bisulphite, on the blanching water is shown (Fig. 5). The
curve is plotted according to pH, since these three agents affect the colora-
tion in proportion to their influence on the pH of the blanch water. The
lowest color values are equal to those of completely blanched potatoes (after
a 60-second blanch).

The influence of two reducing agents, the bisulfite and the nitrite, the
latter at pH 6, is shown (Fig. 6); the activity of the bisulfite is striking.

Dextrose and an amino acid (glycine) have no significant influence,
according to Fig. 7.

A number of other salts have also been investigated, including various
chlorides whose behavior in general resembled the chloride of sodium; vari-
ous sulphates having somewhat less influence; iron salts, which increased
the coloration, or at low hydrogen concentrations showed less decrease

SODIUM NITRITE
SODIUM BISULFITE

1/8 1/2 1% 1 1/2%   3%   4 1/2%   6%

Fig. 6

H₂SO₄
HCl
Na HSO₄
pH CURVE......

Fig. 5
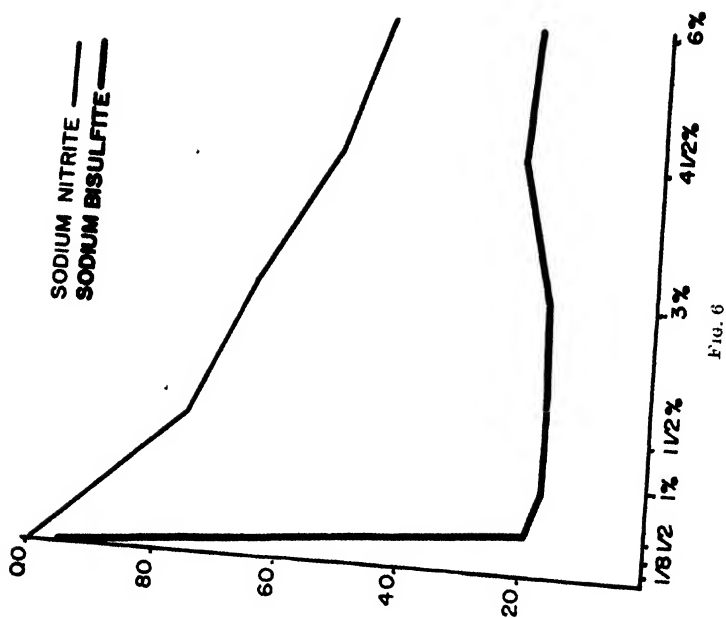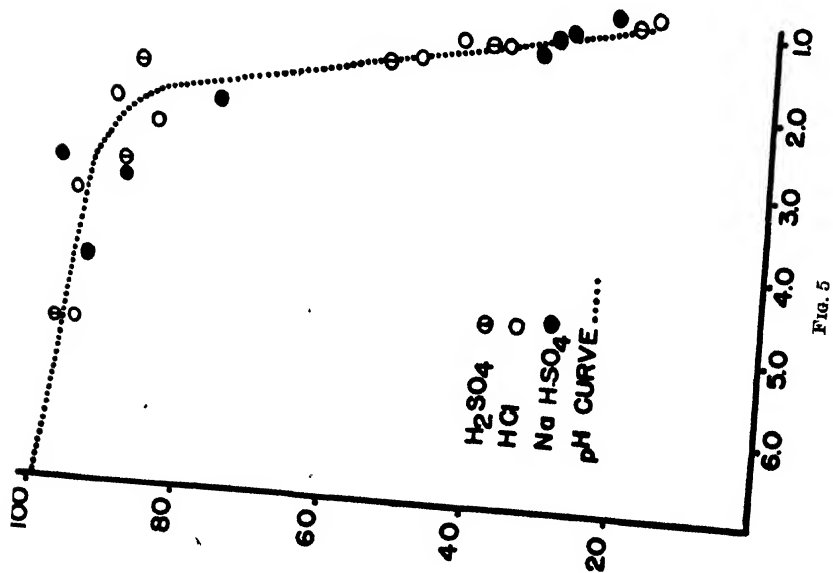
than anticipated at that pH; and alkaline salts, which increased the coloration according to the expected influence of the pH.
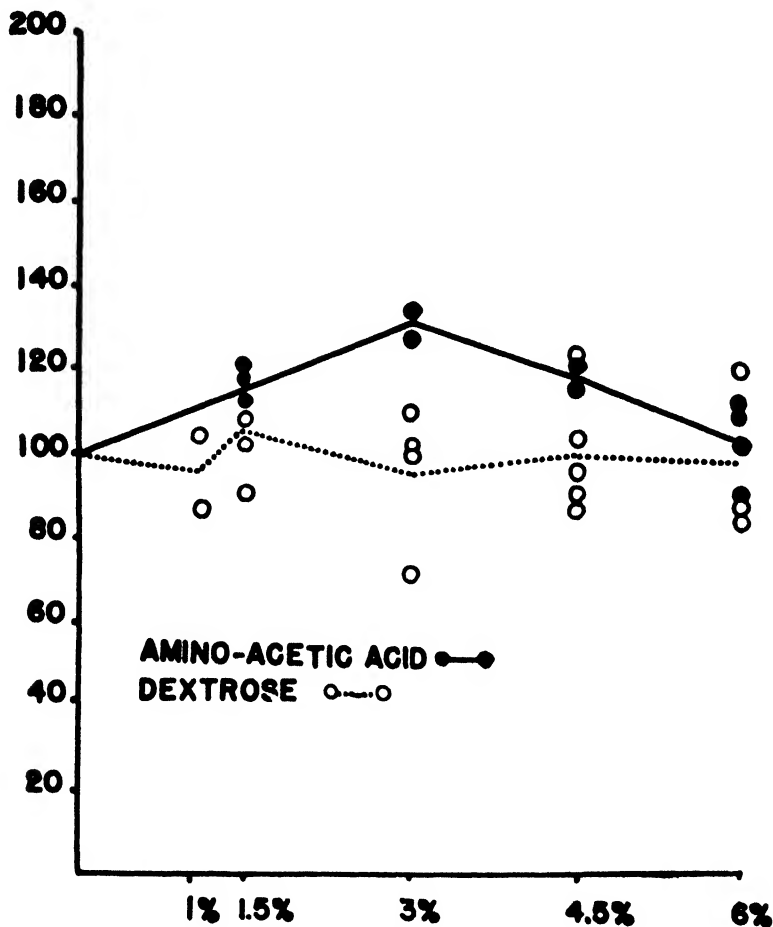
FIG. 7

The direct colorimetric method provides a ready quantitative means for evaluating the color changes in aerated potato juice. Laboratory equipment, such as a blender, a shaker, and a colorimeter, is required. The latter, however, might for practical work be replaced by a set of standard colors.

Since a 50- to 60-second water blanch is sufficient to inhibit coloration, under the conditions investigated, it may be possible to reduce the blanching time to appreciably less than the conventional period now employed.

SUMMARY

The enzymatic discoloration tendency of white potatoes (the so-called "pinking" or "graying") may be quantitatively evaluated by determining

the intensity of color formed in clarified maceration juice under standard conditions.

Wide variations in discoloration tendencies occur among the same batch of potatoes.

The juice coloration, which continues to increase on standing, may be stabilized for at least a half hour in a concentration of 0.1 to 0.05 M sodium azide.

The color formed is in inverse relation to the blanching time, all color formation being halted after a 50- to 60-second blanch in boiling water.

The direct colorimetric method may be employed to determine the influences of various agents in the blanching water.

The presence of acid and sulfite greatly increased the speed of enzyme inactivation by the blanch. Nitrite and various chlorides, particularly the chloride of sodium, also speeded blanching efficiency; sulfates were somewhat favorable; sugars and amino acids were indifferent; and iron and alkali salts were unfavorable.

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BACTERIOLOGICAL INVESTIGATION ON SPOILAGE OF WINTER HERRING DURING STORAGE

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Numerous investigations on fish spoilage have been carried out in recent years. This problem has been attacked from a bacteriological as well as a chemical point of view. Owing to the diversity of methods used and the variety of fish employed, correlation of results is often difficult. Considering the different aspects of the problem of fish spoilage much research has yet to be done before a firm scientific foundation can be established.

In a previous paper Aschehoug and Vesterhus (1943) reported their results of an investigation on the bacterial flora of fresh winter herring. This investigation represented the first phase of a more extensive study of the bacterial flora of winter herring during storage.

Problems concerning the keeping quality of herring are of particular interest in Norway owing to the great economic importance of the winter herring fisheries in that country. During a short season, covering the first months of the year, these fisheries represent an average catch of about 370,000 tons of herring. A great part of the fish is exported iced and salted; a small portion is used in the canning industry for the packing of kippered herrings; while the bulk of the catch is used as raw material for the manufacture of herring oil and herring meal.

LITERATURE REVIEW

Hunter (1920a, 1920b, 1922) studied the decomposition of salmon from a bacteriological viewpoint. For this purpose total counts of bacteria were determined and the types of organisms present in decomposing fish were studied. He reported a total count of 155 million per gram in the muscular tissue of salmon after 96 hours at temperatures between 10 and 21°C. (50 and 69.8°F.). His investigation covered a special study of the organisms responsible for "foul" odors or indol in fish infusions. According to his results bacteria responsible for the decomposition of salmon have their natural habitat in sea water. No sporeforming bacteria were encountered.

Fellers (1926) likewise investigated the bacteria concerned in the spoilage of raw salmon. According to his view spoilage was apparently not due to anaerobic bacteria which were present in small numbers. The penetration of bacteria into the fish was dependent upon such factors as size of the fish, methods of handling, and temperature of storage. Of the organisms isolated, aerobic nonsporeforming bacteria represented 36.5 per cent, cocci 31 per cent, most of the rest being aerobic, asporogenic, chromogenic bacilli.

Sanborn (1930), studying marine bacteria and their relationship to the decomposition of fish, examined particularly the proteolytic power of certain types belonging to the following genera: *Micrococcus, Flavobacterium,* and *Achromobacter.* According to his findings marine bacteria are consistently present on and in fresh fish as well as in smoked and iced fish. These organisms are often psychrophilic and possess proteolytic properties.

Stewart (1934) examined the bacterial flora of different species of market fish, which were seven to eight days old, and reported that the same flora was represented in the slime of the stored fish as isolated from fresh fish. Of the organisms studied the greatest number belonged to the genus *Achromobacter.* The remaining were *Flavobacterium, Micrococcus,* and *Pseudomonas.* A probable agent in decomposition was *Achromobacter pellucidum,* which occurred with a high degree of frequency and possessed marked proteolytic properties.

Griffiths and Stansby (1934) tried to correlate chemical tests and bacterial counts with the degree of freshness of market haddock. They found that counts of about one million bacteria per gram indicated that the fish were stale and no longer in a marketable condition. Fish fillets, on account of their greater initial contamination, spoiled more rapidly.

Kayser (1937) examined the bacterial flora of marked fish, and also included in his studies organisms present in fish kept at different temperatures for various lengths of time. According to his findings gram-negative rods, later proved by Schönberg (1937) to belong to the photobacteria, were demonstrated with a high degree of consistency.

As a result of studies concerning fish spoilage Schönberg (1938) states, in accordance with other investigators, that autolysis is negligible in comparison with spoilage by bacteria. He calls attention to the constant presence of luminous bacteria, especially *Bacterium phosphorescens,* on marine fish and maintains that these organisms are of no importance during subsequent spoilage of the fish. According to his view *Pseudomonas fluorescens liquefaciens* and members of the genus *Flavobacterium* are the most important spoilage agents on account of their proteolytic properties and their ability to grow at low temperatures.

For the purpose of investigating the distribution of bacteria in fresh and stored fish Lücke and Frercks (1940) examined numerous muscle samples excised from cod and made bacterial counts from different sections of the fish. According to their findings the highest counts were always obtained right under the skin in samples from fresh fish as well as fish in decomposition. The infection does not take place via the blood but by penetration of bacteria through the skin into the flesh.

Tarr (1939) studies fish spoilage from a chemical and bacteriological point of view. Being especially interested in the bacterial reduction of trimethylamine oxide, he examined for this purpose 30 different microorganisms which were *Achromobacter, Flavobacterium, Micrococcus,* and yeast, all isolated from spoiling fish in various stages of decomposition. Of these cultures only three were able to reduce trimethylamine oxide, a result which indicates that the use of trimethylamine determination alone as a criterion of freshness of fish must be considered with particular caution.

Watson (1939) likewise was interested in the trimethylamine oxide-reducing bacteria, and studied for this purpose chiefly members of the genus *Achromobacter* which, according to his findings, is chiefly responsible for the spoilage of cod muscle at low temperatures. Some 200 species of facultative anaerobes were studied in cod muscle press juice, besides aerobic types belonging to the genera *Flavobacterium* and *Achromobacter*. He concludes that the reduction of trimethylamine oxide is not a function of the bacterial population since only certain types of bacteria are capable of reducing trimethylamine oxide. He also found that obligate anaerobes are of no importance in the initial stages of fish spoilage.

Literature concerning chemical and bacteriological changes in fish muscle during storage is well reviewed by Notevarp, Hjorth-Hansen, and Karlsen (1942) and Hjorth-Hansen (1943). These investigators concerned themselves chiefly with the chemical side of these problems.

Kimata (1942) made microbiological studies on the spoilage of "semi-dried fishes," examining 181 cultures isolated from fish in decomposition. The organisms were, according to frequency of occurrence, *Micrococcus*, nonsporeforming, and sporeforming rods. The nonsporeforming organisms belonged to the genera *Achromobacter, Flavobacterium,* and *Pseudomonas*. The spoilage of the "semi-dried fishes" is attributed chiefly to the action of cocci and nonmotile rods, thus being different from the spoilage of fresh fish which is principally acted on by the nonsporeforming, motile, water bacteria belonging to *Pseudomonas, Achromobacter,* and *Flavobacterium*.

Thjötta and Sömme (1942) made an extensive bacteriological examination of 19 species of fish including sea fish as well as fresh-water fish taken from different localities in Norway. In all 379 bacterial strains from the skin, gills, and intestines of freshly captured fish were classified according to Bergey (1939) and represented seven families and 16 genera.

Aschehoug and Vesterhus (1943) made a study of the normal bacterial flora of freshly caught winter herring for the purpose of examining the distribution of the initial flora of the fish. This investigation, being the first phase of an extensive study of the bacterial flora of the same fish during storage, was carried out during two winter fishing seasons in order to obtain an average of the normal bacterial flora of fresh fish. According to their findings the fish flesh is sterile. From the slime, gills, and intestines 272 organisms were isolated, and their characteristics were studied in detail. There was no special qualitative difference in the flora from the various sources. The following five genera were represented in the slime and gills: *Pseudomonas, Achromobacter, Flavobacterium, Micrococcus,* and *Proteus*. In the intestines only three of the mentioned genera, *Achromobacter, Pseudomonas,* and *Micrococcus,* were demonstrated. Of potential importance for the keeping quality of the fish was the demonstration of different strains of *Achromobacter* and *Flavobacterium* possessing strong proteolytic properties.

As for the occurrence of strict anaerobes, this question was also investgated and gave a negative result, which goes to prove that organisms of this type do not represent the normal initial flora of freshly caught winter herring.

EXPERIMENTAL PROCEDURE

The purpose of the present investigation was, as outlined in a preceding paper (1943), to continue the first study of the bacterial flora of fresh winter herring by making a systematic study of the bacteria present during storage of the fish under known conditions. Having an intimate knowledge of the previous history of the fish and of the distribution and kind of bacteria originally present, it was intended to follow the different stages of the spoilage process by establishing the types and characteristics of the organisms responsible for decomposition of the fish and comparing them with the initial flora. Of special interest was an examination of the tri-methylamine oxide-reducing power of the isolated strains.

The investigation also included attempts to isolate strict sporeforming anaerobes from spoiled fish, a question of considerable importance to the sterilization problem in the canning industry owing to the highly heat-resistant spores belonging to these groups of organisms.

Collection and Storage of Samples: The raw material used for the present investigation was collected during the winter herring fisheries by the writers while at the fishing grounds. As it was intended to get a cross section of the normal bacterial flora of the fish, the investigation was extended to a period of two years, thus covering two fishing seasons. The fish caught in the beginning of March on the west coast of Norway were in the spawning period and represented an average quality of those used in the canning industry, having a fat content of eight to 10 per cent. The catches, including four lots of fish, were taken on three fishing banks. The live fish were sampled as they came from the nets and transported imme-diately to the laboratory in lots of about 44 pounds (20 kg.) in well-covered wooden cases, one lot being transported in live condition in barrels with fresh sea water. As described in the previous paper, the same lots of fish were used for examination of the initial bacterial flora in the slime, gills, and intestines of the fish as for a study of organisms present in the fish during subsequent storage.

The fish were stored outdoors, in uncovered, wooden cases, the two first lots at about 1°C.(33.8°F.), the others at somewhat higher temperature, varying from 1 to 8°C.(33.8 to 46.4°F.). According to usual canning practice, herring used in the industry is stored either aboard the fishing boats or in the factories, the temperature varying from 0 to 10°C.(32 to 50°F.) depending upon weather conditions.

The decomposition of the four lots occurred very slowly, no advanced spoilage being noticed even after 11 days' storage. It was therefore judged necessary to supplement the investigation with a study of bacteria from herring taken from the industry and stored under usual industrial con-ditions. For this purpose two lots of fish (No. 5 and 6) were stored out-doors in a herring-meal plant, lot No. 5 in wooden cases and lot No. 6 parallel in bulk for nine days at about 2°C.(35.6°F.). In these lots the trimethylamine oxide reduction was followed by regular analyses per-formed simultaneously with, the bacteriological examinations.

Determination of Viable Bacteria in Herring During Storage: During storage of fish, bacteria will penetrate from the slime through the skin into the fish flesh and from the gills via the blood into the blood channels.

As a means of measuring the bacterial invasion the procedure usually adopted is to remove aseptically muscle samples right under the skin for bacterial counts, the same area of muscle in each fish being sampled. It is presumed that the changes occurring in a lot of fish during storage will proceed with approximately the same rate when the origin of the fish is the same and the fish are stored under the same conditions for the same length of time.

In order to reduce the error owing to individual variation in the bacterial number of the fish, an average sample from five fish was used for each examination. This procedure would secure a certain margin of safety according to results from a previous study with six different lots of herring including 24 individual fish.

The following technique was adopted for sampling of the fish. In order to remove muscle samples as aseptically as possible the surface of the fish was first cleansed with absorbent cotton, moistened with alcohol to remove slime and scales, and afterwards flamed. Under aseptic conditions samples of about 10 gm. of muscle were excised with a sterile scalpel from each fish after the skin had been removed with sterile forceps. The muscle samples were taken from the right and left dorsal side of the fish after removal of the surface layer and transferred to a sterile Petri dish. With sterile scissors the fish flesh was clipped finely and was thoroughly mixed, after which about five-gm. samples were transferred to sterile flasks with 95 ml. of physiological salt solution. A maceration of the tissue and thorough distribution of the bacteria were facilitated by the use of glass beads in the flasks. An aliquot part of the suspension was, after suitable dilution depending upon the presumed bacterial count, transferred to the different media for counting in Petri dishes and Burri tubes according to the standard methods for determination of viable bacterial counts of aerobic as well as anaerobic organisms.

After preliminary experiments concerning the most suitable media for the development of bacteria from fish, ordinary plain nutrient agar and gelatin were used, besides fish agar and gelatin, the fish media offering no special advantage except for the study of luminous bacteria, where an extract of soft herring roe added to ordinary nutrient broth greatly favored the development of, e.g., *Pseudomonas phosphorescens.*

The NaCl concentration of the media was 2.5 per cent.

At the outset counts were made both at 37 and 22°C.(98.6 and 71.6°F.). As it was found that the predominant types of fish bacteria usually failed to develop and multiply at the higher temperature, determinations of the 37°C. counts were discontinued. Duplicate plates and tubes were in all cases used, and yielded counts that checked each other rather closely.

As for the search of obligate anaerobes about one-gm. samples of the minced fish muscle were transferred directly into cooked meat medium and liver medium, the tubes being kept at 37 and 22°C. in Zeissler's vacuum jars to secure complete anaerobiosis.

The results of the determination of viable bacterial counts at 22°C. are summarized (Tables 1 to 6), including results of the organoleptic examination of the fish. A possible correlation between the bacterial count and the stage of spoilage was the main purpose.

TABLE 1
Bacteria in Winter Herring, Lot 1, Stored at 1°C.(33.8°F.)

Storage of fish	Organoleptic examination of fish	Average bacterial count per gram at 22°C.(71.6°F.)	
		Aerobes	Anaerobes
days			
0	Normal	8	0
2	Normal	73	700
3	Normal	150	400
4	Normal	1,000	100
5	Strong, fishy odor	1,000	100
7	Strong, fishy odor	805	100
10	Strong, fishy odor	7,600	100
11	Strong, fishy odor	277,500	......
12	Strong, fishy odor	177,500	......

TABLE 2
Bacteria in Winter Herring, Lot 2, Stored at 1°C.(33.8°F.)

Storage of fish	Organoleptic examination of fish	Average bacterial count per gram at 22°C.(71.6"F.)	
		Aerobes	Anaerobes
days			
0	Normal	8	0
1	Normal	150	200
2	Normal	100	100
3	Normal	1,000	100
4	Strong, fishy odor	1,000	100
6	Strong, fishy odor	37,250	2,500
9	Strong, fishy odor	2,250	700
10	Strong, fishy odor	127,500	......
11	Strong, fishy odor	1,550,000	......

TABLE 3
Bacteria in Winter Herring, Lot 3, Stored at Temperatures Varying From 1 to 8°C.(33.8 to 46.4°F.)

Storage of fish	Organoleptic examination of fish	Average bacterial count per gram at 22°C.(71.6°F.)	
		Aerobes	Anaerobes
days			
0	Normal	21	......
2	Normal	350	0
3	Normal	11,820	100
5	Normal	140,200	1,000
6	Stale odor	788,400	......

TABLE 4
Bacteria in Winter Herring, Lot 4, Stored at Temperatures Varying From 1 to 8°C.(33.8 to 46.4°F.)

Storage of fish	Organoleptic examination of fish	Average bacterial count per gram at 22°C.(71.6°F.)	
		Aerobes	Anaerobes
days			
0	Normal	0	0
2	Normal	5	3
4	Normal	6,000	500
6	Stale odor	100,000	1,200

TABLE 5

Bacteria in Winter Herring, Lot 5, Stored at 2°C.(35.6°F.)

Storage of fish	Organoleptic examination of fish	Trimethylamine oxide-N	Average bacterial count per gram at 22°C.(71.6°F.)	
			Aerobes	Anaerobes
days		*mg./100 gm.*		
2	Normal	55	3,000	160
4	Strong, fishy odor	....	27,200	75
6	Stale odor	24	204,000	15,500
9	Putrid	4	18,870,000	166,700
11	Putrid	0	7,625,000	1,007,500

TABLE 6

Bacteria in Winter Herring, Lot 6, Stored at 2°C.(35.6°F.)

Storage of fish	Organoleptic examination of fish	Trimethylamine oxide-N	Average bacterial count per gram at 22°C.(71.6°F.)	
			Aerobes	Anaerobes
days		*mg./100 gm.*		
2	Normal	55	3,000	160
6	Stale odor	29	520,000	72,500
9	Putrid	2	20,900,000	575,000
11	Putrid	0	10,950,000	2,025,000

The bacterial counts remained very low during the experimental period (Tables 1 and 2), the storage temperature being 1°C. Only after 11 days was a proliferation of bacteria perceptible, but no signs of advanced spoilage were noticed. In the two next lots, stored at temperatures varying from 1 to 8°C., the increase in bacterial counts was more pronounced, but even here relatively low values of bacterial numbers were obtained. Quite a different result is expressed (Tables 5 and 6), including the two lots stored under regular industrial conditions. Here advanced spoilage occurred after six days at 2°C., the fish having a stale odor and showing other signs of decomposition. It is of interest to note that at this time the trimethylamine oxide was reduced to about half of that originally found.

As for the presence of obligate anaerobes all the tubes of deep meat medium and liver medium, inoculated with samples of fish muscle from fish withdrawn at regular intervals during the storage period simultaneously with the determination of bacterial counts, remained sterile. This result is of interest as obligate anaerobes are the organisms possessing the most heat-resistant spores and are consequently of importance as contaminants during the processing of fish in the canning industry.

The rôle of the intestines as sources of infection during spoilage of fish has been investigated by many workers. From the literature it is apparent that the number and kind of bacteria present in the intestines of freshly caught fish show great variations. According to earlier investigation of Aschehoug and Vesterhus (1943) the bacterial flora in the intestines of fresh herring included three of the genera demonstrated in the slime and gills of the fish, *Achromobacter, Pseudomonas,* and *Micrococcus.*

It was also of considerable interest to evaluate the bacterial number in the intestinal tract of fresh herring and in fish undergoing decomposi-

tion. For this purpose the stomach and intestines were removed aseptically, clipped in sterile Petri dishes, and samples of about three gm. from each fish were transferred to flasks with sterile physiological salt solution for shaking, subsequent plating, and transferring in Burri tubes.

TABLE 7

Bacteria in Intestines of Fresh Herring

Lot No.	Sample	Average bacterial count per gram at 22°C.(71.6°F.)	
		Aerobes	Anaerobes
Lot 1, including 31 fish................................	Stomach	4,300	160
	Intestines	9,200	1,900
Lot 2, including 17 fish................................	Intestinal tract	3,300	350
Lot 3, including 10 fish................................	Intestinal tract	16,800	15

The average bacterial counts of the intestines of 58 individual fish, of which 31 were examined for bacterial number of both stomach and intestines, are shown (Table 7); results of bacterial counts of the intestines of spoiled herring are given (Table 8).

TABLE 8

Bacteria in Intestines of Spoiled Herring

Lot No.	Average bacterial count per gram at 22°C.(71.6°F.)	
	Aerobes	Anaerobes
Lot 1, including 6 fish..	13,500,000	61,000

It is apparent from the data that the bacterial number in intestines of fresh herring is exposed to some variation. In spoiled herring a very marked increase in the bacterial number is noticeable.

In experiments with herring, air-dried outdoors on galvanized screen, a very marked increase in the bacterial number during the dehydration period was demonstrated, not only in the muscle but also in the intestines of the fish (Table 9). This way of preserving herring to be used as feed for silver foxes is customary in Norway.

TABLE 9

Bacteria in Air-Dried Herring During Dehydration

Storage of fish	Trimethyl-amine oxide-N	Sample	Average bacterial count per gram at 22°C.(71.6°F.)	
			Aerobes	Anaerobes
days	*mg. /100 gm.*			
3	29	Muscle	90,000	6,000
18	0	Muscle	12,700,000	290,000
		Intestines	1,000,000	65,000

EXAMINATION OF BACTERIAL FLORA FROM STORED
WINTER HERRING

A systematic classification of bacteria present in the different lots of fish during storage was made after isolation of the most typical colonies present on the agar and gelatin plates used for bacterial counts. The main purpose was to obtain a cross section of the bacterial flora from the different stages of decomposition of the fish. It was of interest to establish whether a definite succession of bacteria was present during the storage period, and if this was the case, to find a possible correspondence between the stage of spoilage and the dominant organisms demonstrated.

For the purpose of classification the most characteristic colonies present on the plates were isolated and purified by several transfers from solid to liquid media, ordinary nutrient broth and plain nutrient agar with 2.5 per cent NaCl being used. When pure cultures were obtained, their morphological characteristics as well as motility in broth were examined and recorded. Other characteristic features necessary for identification purposes were established by the use of differential media, litmus milk, potato, gelatin, etc., and the ability to ferment carbohydrates was tried out in glucose, sucrose, maltose, lactose, and mannitol. Indol production was tested in tryptone broth and nitrate reduction in nitrate broth. Other physiological characteristics, such as chromogenesis and temperature relations, were likewise tested. As a rule the cultures were held at 22°C., which, according to our earlier investigations and in agreement with other workers on fish bacteriology, is the most favorable temperature for growth of most bacteria from fish.

For identification of the various organisms thus examined Bergey's "Manual of Determinative Bacteriology" (1939) was used, but, as already experienced during previous studies on the bacterial flora of fresh winter herring (1943), a classification according to his manual was very difficult, exact identification of the species found often being impossible owing to inadequate descriptions or lack of correspondence in minor details. In order to avoid confusion Bergey's "Manual" has been followed as closely as possible, and the organisms have been grouped in species related to the nearest ones found. For the sake of clarity and in order to facilitate the comparison of the organisms demonstrated in stored winter herring with those already found originally in the same fish when quite fresh, as described in our previous investigation (1943), the same numbers in the various tables have been retained.

Results of Examination: From the different lots of stored winter herring 400 cultures were isolated and classified. A percentage record of the organisms obtained from the different dates showed that no definite succession of groups of bacteria could be established, nor could any qualitative difference in the bacterial flora from the different lots be demonstrated. The prevalence of the genus *Achromobacter* from every sample of fish was marked. The following genera named according to the frequency of occurrence were represented: *Achromobacter, Pseudomonas, Flavobacterium,* and *Micrococcus*. The generic percentages of the total isolated strains were *Achromobacter* 58.5, *Pseudomonas* 33.7, *Flavobacterium* 7.0, and *Micrococcus* 0.8.

TABLE 10

Achromobacter Groups

Details and media	No. 1	No. 2 (a)	No. 2 (b)	No. 2 (c)	No. 4	No. 5	No. 6	No. 7
Morphology	Rods, short, slender, occurring singly.	Rods, short to medium size, occurring singly, occasionally in short chains.			Rods, medium size, occurring singly and in short chains.	Rods, short, thick with rounded ends, occurring singly, occasionally in chains.	Rods, short, stout. varying in size, occurring singly, in pairs and occasionally in short chains.	Coccobacilli, medium size, occurring singly.
Gram stain	÷	÷			÷	÷	÷	÷
Motility	+	+			+	÷	÷	÷
Agar colonies	Grayish, raised, drop-like, glistening, smooth, translucent.	Grayish, slightly raised drop-like, translucent.			Grayish, slightly raised, smooth, glistening.	Grayish, slightly raised, smooth, glistening.	Grayish, raised, glistening, opaque.	Grayish, flat, opaque.
Gelatin stab	No liquefaction.	Liquefaction, crateriform to stratiform.			No liquefaction.	No liquefaction.	Liquefaction, crateriform to stratiform.	No liquefaction.
Broth	Turbid, becoming viscid. Pellicle.	Turbid with ring.			Turbid with ring.	Turbid with ring.	Turbid with ring.	Turbid.
Litmus milk	No change.	No change.			No change.	No change.	No change.	Reduced. Faintly acid.
Potato	No growth.	÷ (a)	+ (b)	+ (c)	Gray, filiform growth.	Rugose.	No growth.	Luxuriant growth
Indol	÷	÷	÷	÷	÷	÷	÷	÷
Nitrate	÷	÷	÷	÷	÷	÷[3]	÷	÷
Glucose	÷[1]	÷[1]	÷[2]	÷	÷	÷[4]	÷	÷
Sucrose	÷	÷	÷	÷	÷	÷	÷	÷
Maltose	÷	÷	÷	÷	÷	÷	÷	÷
Lactose	÷	÷	÷	÷	÷	÷	÷	÷
Mannitol	÷	÷			÷			÷
Relation to oxygen	Aerobic.	Aerobic.	Aerobic.	Aerobic.	Aerobic.	Aerobic, facultative.	Aerobic.	Aerobic.
Number of cultures	27	38	23	8	4	19	10	3
Related to	*Achromobacter guttatum* (Zimmerman) Bergey et al.	*Achromobacter liquefaciens* (Eisenberg) Bergey et al.			*Achromobacter pestifer* (Frankland and Frankland) Bergey et al.		*Achromobacter butyri* Bergey et al.	*Achromobacter ubiquitum* (Jordan) Bergey et al.

Details and media	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13
Morphology	Rods, short, stout, varying in size, occurring singly.	Rods slender, medium size, occurring singly and in chains.	Rods, short, slender, occurring singly.	Rods, small, short, often in chains.	Rods, small, short, occurring singly.	Rods, short, medium size, occurring singly.
Gram stain	+	+	+	Gram variable.	+	+
Motility	+	+	+	+	+	+
Agar colonies	Grayish-white, opaque, smooth, becoming darker.	Whitish-gray, raised, smooth, opaque.	Grayish, becoming darker, smooth, raised.	Slightly rose-colored, raised, opaque, glistening.	Bluish-white, translucent, small, circular, raised.	Grayish-white, glistening, smooth.
Gelatin stab	Liquefaction, stratiform.	Liquefaction, stratiform.	No liquefaction.	No liquefaction.	No liquefaction.	Liquefaction, crateriform to stratiform.
Broth	Turbid.	Turbid.	Turbid with ring.	Turbid, pellicle.	Turbid with ring.	Turbid with ring.
Litmus milk	Reduced. Faintly acid.	No change.	No change.	No change.	Slow reduction.	Reduced.
Potato	Good growth, dirty-white, moist, viscid.	Luxuriant growth, rugose.	No growth.	Good growth, rose-colored.	Good growth, grayish-white.	Good growth, yellowish-brown.
Indol	+	+	+	+	+	+
Nitrate	+	+	+[5]	+	+	+[6]
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+[7]
Relation to oxygen	Aerobic, facultative.	Aerobic.	Aerobic.	Aerobic.	Aerobic, facultative.	Aerobic, facultative.
Number of cultures	15	5	69	2	4	7
Related to	......	......	......	......	......	......

[1] Seven strains positive. [2] Five strains negative. [3] Three strains positive. [4] Three strains positive. [5] Ten strains positive. [6] Three strains negative. [7] Twenty-nine strains negative. [6] Three strains positive. [7] Three strains positive.

The most frequently occurring genus was *Achromobacter,* which included 234 strains. For the classification of this genus the system of Bergey proved to be very inadequate. The isolated strains were therefore grouped in species "related" to those of Bergey, when the main characteristics corresponded. Several organisms could be classified only in groups and no names were given. The numerically most important groups were No. 2 and 10, including each about 30 per cent of the total number of *Achromobacter* identified. The organisms in Group 2 exhibited a very characteristic growth on agar, having grayish, drop-like, translucent colonies. They showed a slow but marked liquefaction in gelatin stabs and may consequently be of importance as a spoilage agent in fish owing to their proteolytic properties. These organisms were very similar to the species *Achromobacter liquefaciens* described by Bergey. Some variations in the growth on potato as well as in glucose and maltose made a subdivision into three groups (a, b, and c) necessary. It is of interest to note that the same organisms, related to *Achromobacter liquefaciens,* had already been demonstrated in relatively great numbers in the slime of the same herring while fresh.

The other numerically important group of *Achromobacter,* No. 10, could not be classified according to Bergey. These organisms were indifferent to carbohydrates and negative in respect to proteolytic power. They may possibly be of no importance as a spoilage agent in decomposing fish. About half of the isolated strains reduced nitrates. This species had not been met with in fresh herring.

Numerically important also was Group 1, including organisms which had already been demonstrated in fresh fish. Macroscopically these bacteria resembled those in Group 2, having the same typical drop-like appearance. They did, however, not liquefy gelatin. A characteristic feature was their ability to make the broth viscid. Except for growth on potato the main characteristics corresponded with that of *Achromobacter guttatum.*

Three other groups of *Achromobacter,* No. 5, 8, and 6, ordered according to frequency of occurrence, were numerically of importance. Group 5 had already been demonstrated in relatively great numbers in fresh herring. The organisms were short, nonmotile rods. They showed a characteristic rugose growth on potato and did not possess proteolytic properties. An identification of this species with any of those in Bergey was not possible. Nor could the organisms grouped in No. 8 be classified according to his system. This species possessed proteolytic properties. A characteristic growth on agar of grayish-white colonies, becoming darker, was noticed as well as a dirty-white growth on potato and a reduction of litmus milk. This species had already been demonstrated from the slime of the fresh fish. As for Group 6 the cultural and physiological characteristics of the organisms classified in this group were almost identical with those of *Achromobacter butyri.* The negative growth on potato of our strains did not correspond, however, with the description of Bergey, nor did the positive test on nitrate reduction.

The rest of the groups were numerically less significant. Group 13, not previously demonstrated in fresh herring, could not be classified according to Bergey. Organisms belonging to this group were motile, short rods

TABLE 11

Pseudomonas Groups

Details and media	No. 1 (a)	No. 1 (b)	No. 2	No. 3
Morphology	Coccobacilli to short, thick rods, occurring singly and in pairs.		Rods, medium size, occurring singly and in pairs.	Rods, short, occurring singly.
Gram stain	÷		÷	÷
Motility	+		+	÷
Agar colonies	Translucent with bluish shine, becoming dark opaque, small, moist, smooth.		Grayish, circular, glistening; medium turning green.	Bluish-white, small, circular, raised.
Gelatin stab	No liquefaction.		Infundibuliform liquefaction, greenish coloration.	No liquefaction.
Broth	Turbid, thin pellicle.		Turbid, pellicle.	Turbid with ring.
Litmus milk	No change.		Alkaline.	Reduced.
Potato	No growth.		Yellow-brown growth.	No growth.
Indol	÷	÷	÷	÷
Nitrate	+	÷	+	÷
H$_2$S	÷	÷	÷	÷
Glucose	÷	÷[1]	+	+
Sucrose	÷	÷	÷	+
Maltose	÷	÷	÷	+
Lactose	÷	÷	÷	÷
Mannitol	÷	÷	÷	+
Relation to oxygen	Aerobic, facultative	Aerobic, facultative	Aerobic.	Aerobic, facultative.
Number of cultures	85	44	4	2
Species	*Pseudomonas phosphorescens.* (Fischer) Bergey *et al.*		*Pseudomonas fluorescens* Migula.	Related to *Pseudomonas non-liquefaciens.*

[1] Two strains positive.

possessing proteolytic properties. They fermented the different carbohydrates and exhibited a good growth on potato.

Of the 400 cultures obtained from stored winter herring the next numerically important genus was *Pseudomonas* of which 135 strains were classified and ordered in three species of which Group 1 included most of the strains. In conformity with our previous classification this group was subdivided (a and b) according to the ability of the strains to reduce nitrates. The organisms were identified with *Pseudomonas phosphorescens*, the typical characteristics of which are recorded (Table 11) and corresponded closely to the description given by Bergey. The bacteria have a very characteristic growth on agar, the colonies possessing a translucent

TABLE 12

Flavobacterium Groups

Details and media	No. 1	No. 2	No. 3	No. 4	No. 5
Morphology	Coccobacilli, medium size, occurring singly.	Rods, medium length, slender.	Rods, medium length, slender.	Rods, small, short.	Rods, small, short, varying in size, occurring singly, occasionally in short chains.
Gram stain	÷	+	(Gram variable.)	÷	÷
Motility	+	+	+	+	÷
Agar colonies	Pale lemon, opaque, flat.	Creamy, raised, glistening, opaque.	Apricot, slightly raised, smooth, shining, viscid.	Yellow, flat, glistening, smooth, viscid.	Yellow, small colonies, pulvinate, smooth, glistening.
Gelatin stab	Liquefied, crateriform to stratiform.	Slow liquefaction, infundibuliform.	Slow liquefaction. Stratiform.	No liquefaction.	No liquefaction.
Broth	Turbid.	Turbid.	Turbid.	Turbid with ring.	Turbid.
Litmus milk	Reduced, faintly acid.	No change.	Reduced.	No change.	No change.
Potato	Dry, wrinkled growth, yellow.	Good growth, slightly yellow.	Yellow growth.	No growth.	Luxuriant, dry, rugose growth.
Indol	÷	+	+	+	+
Nitrate	+	+	+	+[1]	+
Glucose	+	+	+	+	+
Sucrose	+	+	+	+	+
Maltose	+	+	÷	÷	÷
Lactose	+	÷	÷	÷	÷
Mannitol					
Relation to oxygen	Aerobic, facultative.	Aerobic, facultative.	Aerobic.	Aerobic.	Aerobic.
Number of cultures	2	1	1	2	1
Related to	......	*Flavobacterium suaveolens* (Kalantarian a. Petrossian) Bergey *et al.*	*Flavobacterium suaveolens* (Kalantarian a. Petrossian) Bergey *et al.*	*Flavobacterium breve* (Frankland a. Frankland) Bergey *et al.*	......

Details and media	No. 6	No. 7	No. 9	No. 10
Morphology	Rods, medium size, occurring singly.	Rods, small, slender, occurring singly.	Rods, very small, short, occurring singly.	Rods, short, medium size, occurring singly.
Gram stain	+	÷	Gram variable.	+
Motility	÷	+	÷	÷
Agar colonies	Yellow, shining, smooth, dry.	Pale yellow, smooth, shining.	Greenish-yellow, raised, circular, entire, smooth, glistening.	Yellow, becoming dirty, raised, entire, glistening.
Gelatin stab	Slow liquefaction, crateriform to stratiform.	No liquefaction.	No liquefaction.	Liquefaction, crateriform.
Broth	Turbid.	Turbid with ring.	Turbid.	Turbid.
Litmus milk	No change.	Reduced.	No change.	Reduced, slow peptonization.
Potato	Good growth, lemon-yellow, rhizoid, dry.[2]	Yellow growth.	Yellow growth, dry.	Good growth, dirty-yellow, dry.
Indol	÷	÷	÷	÷
Nitrate	+	+	+	÷
Glucose	÷	÷	÷	+
Sucrose	÷	÷	÷	+++
Maltose	÷	÷	÷	÷
Lactose	÷	÷	÷	÷
Mannitol				
Relation to oxygen	Aerobic.	Aerobic.	Aerobic.	Aerobic.
Number of cultures	6	5	9	1
Related to	....	....	....	....

[1] One strain positive. [2] One strain no growth.

bluish shine and later becoming darker in color. Their most characteristic feature is their ability to produce luminescence, which could be demonstrated in broth cultures. An addition of soft herring-roe extract to the broth seemed to have a favorable influence on the light production.

Under strict anaerobic conditions in Burri tubes the organisms showed no growth, but when cultivated on agar plates kept at 50 mm. pressure in vacuum jars, growth could be demonstrated. The organisms were consequently facultative aerobic. ·

Two other species of *Pseudomonas* were demonstrated in stored herring, which numerically were of no importance, namely *Pseudomonas fluorescens* and a species related to *Pseudomonas non-liquefaciens*.

In the genus *Flavobacterium* only seven per cent of the 400 isolated cultures were classified and they were ordered in nine groups. Of these groups No. 9, which was the most numerous, included organisms showing a characteristic growth on agar with greenish-yellow colonies which were smooth and glistening. This organism, tested in gelatin stabs, had no liquefying power, and was also negative in respect to carbohydrate fermentation. As none of the 58 species of *Flavobacterium* described by Bergey corresponded with this organism, it was placed in a special group in our classification. Its presence in stored herring was expected, as it had been encountered in relatively great numbers in the slime from the fresh fish.

The next numerically important group of *Flavobacterium* was No. 6, representing yellow, shining colonies on agar. The organisms possessed proteolytic power but were negative with regard to carbohydrate fermentation. Group 7 included organisms showing no liquefaction of gelatin. Neither of these groups could be classified according to Bergey. The remaining six groups of *Flavobacterium* represented less interest owing to their rarity of occurrence (Table 12).

Only about one per cent of the 400 cultures isolated was *Micrococcus*. These organisms corresponded with Group 7 in our earlier classification. An identification according to Bergey was not possible. The organisms represented greyish-white colonies on agar and possessed no proteolytic power. They had been demonstrated in the slime of the fresh herring.

Determination of Trimethylamine Oxide Reduction of 75 Cultures: It has been shown by different investigators that spoilage of fish is accompanied by a reduction of trimethylamine oxide present in sea fish. This reduction is produced by facultative anaerobic bacteria which are also capable of reducing nitrates, Watson (1939). It was of interest to determine whether the cultures isolated from stored herring were trimethylamine oxide reducers. For this purpose 75 cultures were picked at random from the nitrate-positive types and examined in the following manner:

The medium used was that employed by Watson (1939) and consisted of three gm. meat extract, five gm. peptone, five gm. NaCl, 6.8 gm. KH_2PO_4, 18 gm. Na_2HPO_4 12 aq., and 7.5 gm. trimethylamine oxide dissolved in water, adjusted to pH 6.9 and made up to a liter. This medium was tubed and sterilized. About 0.1 ml. of a fresh culture in ordinary broth of the organism to be examined was inoculated into the test medium and kept under aerobic condition in an incubator at 22°C. for two days. At the end

TABLE 13

Micrococcus

Details and media	Group 7
Morphology..	Spheres, small, occurring singly.
Gram stain..	+
Motility..	÷·
Agar colonies...	Grayish-white, flat. amoeboid.
Gelatin stab...	No liquefaction.
Broth..	Turbid.
Litmus milk..	Reduced.
Potato..	No growth.
Indol..	÷
Nitrate..	÷
Glucose..	+
Sucrose..	+
Maltose..	+
Lactose...	÷
Mannitol...	÷
Relation to oxygen...	Aerobic.
Number of cultures..	3

of this period the whole content of the test tube was transferred to a 50-ml. flask. An aliquot part was distilled with NaOH for an eventual determination of trimethylamine, when a reduction of the oxide had been produced. It is apparent (Table 14) that not all of the strains classified as the same species yielded concordant results.

TABLE 14

Trimethylamine Oxide Reduction

Cultures examined	Number of strains examined	Positive
		pct.
Achromobacter—Group 5..	10	60
Achromobacter—Group 6..	1	0
Achromobacter—Group 8..	4	75
Achromobacter—Group 10..	40	15
Achromobacter—Group 12..	3	33
Achromobacter—Group 13..	4	75
Pseudomonas—Group 1a..	7	0
Pseudomonas—Group 2...	1	0
Pseudomonas—Group 3...	1	0
Flavobacterium—Group 1...	1	0
Flavobacterium—Group 3...	3	0

It is of interest to note that the trimethylamine oxide reducers were all facultative aerobes belonging to the genus *Achromobacter*. Of the species examined Groups 8 and 13 also possessed proteolytic power and are probably the most active agents during decomposition of the fish.

According to our earlier experiments with different species of fish, also including winter herring, the fish tissue is sterile. The bacteria which penetrate into the fish flesh during storage are derived from the slime offering a favorable medium for bacterial development and from the gills, which owing to their blood content also furnish a good medium. According to the present investigation the intestines harbor bacteria which in fresh herring amount to numbers from 3,000 to 20,000 aerobes per gram and considerably fewer anaerobes (about 10 per cent).

There seemed to be no relationship between the amount of undigested food in the stomach and the bacterial number. Winter herring shoaling around the Norwegian coast on their spawning migration in February and March are pelagic fish. Their principal food is plankton. At this season the amount of plankton in the ocean is small, compared with that in the spring and summer. As the herring in the spawning season move in pure ocean water on the coast, a high bacterial number in the intestinal tract would not be expected.

Other investigators of fish bacteriology—Hunter (1920a) and Fellers (1926)—state that the digestive tract of migrating salmon was usually sterile. These results are confirmed by Blake (1935) who reports that the stomach and intestines of fasting fish are frequently bacteria-free.

During the storage period of the herring bacterial invasion will occur not only through the skin and via the gills into the fish flesh but also from the intestines into the tissues. A determination of the bacteria in the intestines from spoiled herring yielded about 14 million aerobes per gram and also in the intestines of air-dried herring, high amounts of bacteria.

The decomposition of fish is effected by enzymatic changes and by the influence of bacterial action. The autolysis is, according to the view of most investigators, negligible in comparison with spoilage by bacteria. An evaluation of the bacterial counts in six lots of herring during storage showed that the initial flora consisted of aerobes, the anaerobic bacteria appearing later during the decomposition process. A reduction of trimethylamine oxide accompanied the proliferation of the latter group.

As for the bacteria isolated during the decomposition period a comparison of the flora from the different dates revealed that there was no change in the qualitative composition of the flora. The following genera were represented according to frequency of occurrence: *Achromobacter*, *Pseudomonas*, *Flavobacterium*, and *Micrococcus*. These organisms were expected as they had already been demonstrated in the same fish, while fresh. Earlier studies had revealed that there was no special bacterial flora typical for Norwegian winter herring.

The genus demonstrated with the highest degree of frequency was *Achromobacter*, while in fresh herring the *Pseudomonas* had been prevalent. Evidence seems conclusive that the most active agents responsible for decomposition of fish belong to the genus *Achromobacter*. Special mention must be made of the species grouped in No. 8 and 13 (Table 10), which were at the same time active trimethylamine oxide reducers and gelatin liquefiers. They were, however, numerically not dominating. Other strains possessed only the trimethylamine oxide-reducing power. It is of interest

to note that organisms possessing this property were all facultative aerobes, a finding which is in conformity with the result of other investigators. One of the most numerous groups related to *Achromobacter liquefaciens,* possessing proteolytic properties, had been encountered in great numbers in the slime and gills of the fresh fish.

The organisms belonging to the numerically next important genus were *Pseudomonas* of which most of the strains were identified with *Pseudomonas phosphorescens.* This organism, a typical sea-water bacterium, can be isolated in great numbers from the surface of fresh herring, showing luminescence after only one day's storage. *Pseudomonas phosphorescens* belongs to the groups of bacteria that produce the phenomenon of phosphorescence of the sea. The organisms probably do not take active part in the decomposition process of the stored herring. Their presence in decomposing fish has also been demonstrated by Schönberg (1937). This investigator emphasized especially the importance of *Pseudomonas fluorescens* as a spoiling agent in stored fish. We could identify only four of the 135 strains classified with *Pseudomonas fluorescens.* This organism does not belong to the normal flora of ocean water but indicates pollution from the shores.

The remaining genera, *Flavobacterium* and *Micrococcus,* were of minor importance owing to rarity of occurrence. Most of the species of *Flavobacterium* had already been demonstrated in the fresh fish.

The search for sporeforming, obligate anaerobes in the samples of stored herring gave in all cases a negative result. Other investigators have arrived at the same conclusions with other species of fish, with the exception of fish feeding on the bottom, such as haddock—Shewan (1938). The presence of sporeforming, obligate anaerobes in canning plants is important since the spores of these organisms offer great resistance to heat. According to our previous investigations the slime, gills, and intestines of the freshly caught fish did not harbor obligate anaerobes. Their presence in the canning operations must therefore be attributed to incidental contamination during transport of the fish and to unhygienic conditions in the canning plant itself. This result confirms the conclusions already drawn in our previous paper and is of interest for the canning industry, as it throws light on the importance of sanitary quality of the raw product to be canned and on hygienic conditions in the factories.

Considering the determination of the bacterial counts in the six lots of fish in the present investigation, attention has been already called to the difference in results between Lots 1 to 4 (Tables 1, 2, 3, and 4) and the two next lots (Tables 5 and 6). While the bacterial increase in the four first lots occurred very slowly, the next lots underwent a rapid decomposition and showed high bacterial counts. This is explained by different factors, such as the initial bacterial infection and the transport and storage conditions of the fish.

Concerning the first four lots, the fish were sampled as they came from the nets and proved to be almost bacteria-free. All precautions were taken during the transport to avoid external infection, as the planned bacteriological examination included a study of the bacteria present on the fish straight from the sea and of the same fish during subsequent storage.

The fish were stored in lots of about 20 kilograms in flat wooden boxes. The lower layers were consequently not exposed to any great pressure and the fish were not damaged. During storage in uncovered cases the fish had free access to air. The upper layers would consequently be partly dehydrated and the skin less penetrable for bacteria. It is a general experience in the herring oil and meal industry that decomposition of fish is accelerated by storage in piles a few meters deep, while a spreading of the fish over a large area retards the decomposition.

The above factors account for the poor development of bacteria during storage in the first four lots of fish. It was clearly shown that spoilage of winter herring is greatly retarded when the initial bacterial infection is low. This reflects the importance of the hygienic condition of the raw product in the industry.

With regard to the next two lots (No. 5 and 6), the raw material of which was procured from the herring-meal industry, spoilage occurred within about six days at a storage temperature of 2°C. This was in accordance with expectancy, as we had already carried on experiments with storage of winter herring of the regular quality used in the canning factories, Aschehoug and Vesterhus (1940). The initial bacterial number in winter herring used in the industry usually varied from a few thousand to hundred thousands of bacteria per gram of fish.

According to usual shipping practice, winter herring is transported in bulk on board fishing boats or in wooden cases holding about 100 kilograms. Rough handling of the fish will result in broken skin and tissues, favor the distribution of organisms present in the slime and on the surface of the fish, and allow them to penetrate into the flesh. When the fish are transported in bulk, the lower layers will be exposed to a great pressure which will cause contents of the gut to be pressed out. Since the fish are captured during the spawning season, ordinary roe and soft roe also will be partly pressed out and mixed with blood and slime. All these substances together constitute an excellent medium for bacterial development.

The best proof of the importance of sanitary transporting of winter herring was obtained during the last years of World War II, when the fish were transported bulked under unfavorable warm weather conditions in small vessels requiring exceptionally long time for transit. It consequently happened that lots of fish arrived at the factories in a poor, partly decomposed condition and had to be discarded as unfit for canning purposes.

Lumley, Piqué, and Reay (1929) made an extensive study of the various sources of infection during transport of fish at sea. They demonstrated by bacterial counts the result of washing and gutting of the fish and also showed that the infection of the surfaces of the fish can be kept at a minimum by avoiding contact with infected decks, deck fittings, and baskets. They also called attention to the importance of boxing the fish and concluded that pressure and much handling produced flabby fish. Lücke and Schwartz (1937) likewise studied the transport conditions for fish from a bacteriological point of view and demonstrated a lower bacterial number in fish transported in boxes than in bulk. They also emphasized that pressure on the fish would favor the distribution of bacterial infection. The

results of these investigators are in agreement with our findings and seem to substantiate our explanation of the exceptionally low initial bacterial count of the first four lots of herring.

SUMMARY

A bacteriological study was made on four lots of winter herring collected during two winter fishing seasons and transported under aseptic conditions to the laboratory. Two lots were stored at about 1°C., the other at temperatures varying from 1 to 8°C. Samples were withdrawn regularly for bacterial counts and for a systematic study of the predominant organisms present during storage. As the initial flora of the same herring straight from the sea had already been established and reported in a previous paper by Aschehoug and Vesterhus (1943), a comparison between the organisms responsible for spoilage of the fish and the bacteria originally present was possible. The bacterial counts remained low even in the fish stored at the higher temperature, and decomposition occurred very slowly, a fact which was attributed to an unusually low initial bacterial infection and unfavorable conditions for bacterial proliferation during storage. The investigation was supplemented with two lots of fish of the ordinary quality used in the herring industry which underwent spoilage in the expected period. Samples were likewise withdrawn regularly for bacterial counts, for a study of the flora, and also for trimethylamine oxide determinations.

Winter herring move in schools off the Norwegian coast in clean ocean water, their principal food being plankton. Bacterial counts of intestines of 58 fresh herring were made, as well as of stomachs of 31 fish, giving figures varying from 3,000 to about 20,000 bacteria per gram. Bacterial counts of intestines of spoiled herring (six fish) were also established and showed a high increase in bacterial numbers.

A study of the predominant organisms isolated from the stored herring included 400 cultures, which were classified according to the system of Bergey. There was a good correspondence between the organisms already demonstrated in the herring while fresh, and those isolated from the stored fish, the same genera being demonstrated. While *Pseudomonas* was the predominating genus in fresh herring, *Achromobacter* was demonstrated with the highest frequency in the stored fish. According to frequency of occurrence the following genera were represented: *Achromobacter* 58.5, *Pseudomonas* 33.7, *Flavobacterium* 7, and *Micrococcus* 0.8.

Evidence seems conclusive that decomposition of fish is due mainly to organisms belonging to the genus *Achromobacter*. Among the 234 strains of *Achromobacter* were active trimethylamine reducers (30 per cent of the strains examined) and also proteolytic types, which are responsible for the production of volatile bases and of protein breakdown. These organisms were facultative aerobes. It is of interest to note that the most active species were not the most numerous.

The genus *Pseudomonas* included mostly *Pseudomonas phosphorescens,* which can be isolated in great numbers from luminous fish.

The question concerning the occurrence of sporeforming obligate anaerobes in stored fish was given special consideration owing to the importance of the heat-resistant organisms of these groups in the canning industry. All attempts to isolate obligate anaerobes gave negative results.

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NIACIN, RIBOFLAVIN, AND THIAMIN STUDIES ON DEHYDRATED PORK LOAVES [1]

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The problems of transportation and meat shortage associated with the lend-lease program of the recent war have given an impetus to research studies on dehydrated meats. Since the nutritional investigations on dehydrated meat have been of such recent date, few reports have appeared in the literature.

The report here presented includes studies initiated in 1943 on the effects of temperature, container, and incorporation of soya flakes on the niacin, riboflavin, and thiamin contents of dehydrated pork loaves during storage.

Rice, Beuk, and Robinson (1943) reported that dehydrated pork retained little thiamin after several weeks' storage at 48.9°C.(120°F.) and that the thiamin could be stabilized to an appreciable extent by the inclusion of a mixture consisting of cereals, milk, tomato paste, and bone meal. The unsupplemented product retained 15 per cent of the original thiamin after one week's storage at 120°F., as compared with a 74 per cent retention of thiamin in pork supplemented with non-meat solids to the extent of 33 per cent. In later studies, Rice and Robinson (1944) indicated further that at temperatures up to 37.2°C.(99°F.) there is little or no loss of niacin or riboflavin over a period of 219 days. Thiamin decreases more rapidly, showing some loss at 26.7°C.(80°F.). After 219 days' storage the thiamin retention in dehydrated pork is 29 per cent, while at higher temperature there is almost complete destruction of thiamin. Rice, Beuk, Kauffman, Schultz, and Robinson (1944), in their preliminary studies, have shown that dehydrated pork and dehydrated eggs stored at or above temperatures of 37°C.(98.6°F.) lose thiamin more rapidly than certain other foods, such as dried skim milk and cereals. Dehydrated mixtures of 67 per cent pork and 33 per cent cereals or milk solids lose thiamin much less rapidly than dehydrated pork alone. The thiamin loss of the dehydrated pork was found to be somewhat related to the moisture level over the range of 0 to 6 per cent. Thiamin retention was not affected by storage in vacuum, nitrogen, or carbon dioxide rather than air.

Products rich in carbohydrate material were shown to be the most effective stabilizing agents studied. The authors postulated that the sta-

bilizing action of these substances may be due to chemical or physical properties which enable them to inhibit the action of water on thiamin, or if the destructive action is due to enzymic activity, the excess of carbohydrate or some substance occurring with it might inhibit the reaction between thiamin and the enzyme.

The loss of thiamin was also shown to be real and not merely a "binding" of the vitamin as indicated by comparable results obtained by the thiochrome and rat-growth methods of determination.

In studies carried out principally by the Department of Agriculture's Research Center at Beltsville, Maryland (1944), it was also observed that thiamin in dehydrated pork is greatly affected by temperature, the loss being nearly 100 per cent after storage for eight weeks at 43.3°C.(110°F.), compared with a 33 per cent loss at 21.1°C.(70°F.). Niacin losses are not significant. These workers find the laminated lead-foil bag to be the best substitute for the properly sealed can.

EXPERIMENTAL PROCEDURE

Formula and Preparation of Pork Loaves: Ground pork shoulder was the meat chosen for this study since this cut would be typical of that used for dehydrated pork. Soya flakes were used as the extender replacing a part of the pork shoulder in the "extended" pork loaf.

The following basic formula was used, Hotaling and Fenton (1945):

SOYA-EXTENDED LOAF

Pork	454	parts by weight
Soya flakes	65	parts by weight
Spray-dried, whole egg	12	parts by weight
Ground onions	18	parts by weight
Sodium glutamate	2	parts by weight
Sage	0.4	part by weight
Pepper	0.6	part by weight
Salt	9	parts by weight

The control loaf was of the same formula except that the soya flakes were replaced with pork.

In order to obtain a sufficient number of samples for the storage tests it was necessary to prepare four different lots of the meat loaves. From the standpoint of the accompanying data, it must be noted that the different lots showed some variance owing to the slightly different treatment of the four lots of meat loaves. There were variations in the time of drying, the order in which the ingredients were mixed, the fat contents, and the separation of fat from the last three lots with subsequent readdition after dehydration. Then, too, the pork itself may have varied in original vitamin content. Marked changes and trends in vitamin retention under the conditions of study are evident, however, in spite of the above-mentioned variations.

The procedure finally developed, used in general for the last three lots, is as follows: The partially frozen pork was placed in the steam-jacketed kettle of a Hobart food mixer. After about 45 minutes of intermittent mixing the meat had reached the desired precooking temperature of 73.9°C.

(165°F.). The meat was held at this temperature for a 30-minute period. During this period the fat and moisture partially separated from the meat. The aqueous and fatty layers were removed, were allowed to separate as far as possible, and the aqueous layer was reincorporated with the meat. The ground onions, spices, and soya flakes were added to the meat immediately before dehydration. The mixes were dehydrated in specially constructed, forced-draft, cabinet-type dehydrators, designed and constructed by Dr. F. B. Wright, who kindly made them available for this study. Each batch was distributed as evenly as possible over trays consisting of wooden frames with plastic screen bottoms. The layers were about one-half to one inch deep. The thermostats were set for a maximum of 68°C.(154.4°F.) and toward the end of the drying period this maximum was reached and maintained. Owing to the fineness of the ground meat, stirring of the products was found necessary to ensure uniform drying. This was accomplished whenever samples were taken (about every 1½ hours) to follow the moisture content. The Bidwell-Sterling toluene distillation method for moisture analysis was sufficiently accurate for the purpose required here—namely, a rapid method which could be applied to the products while dehydration progressed. Twenty-gram samples were taken directly from the trays and in about three quarters to one hour the moisture analysis could be obtained. The results obtained by this method were found to check with the five-hour vacuum method at 100°C.(212°F.). The total drying time required to give products below 10 per cent moisture was about seven to eight hours. The powdered egg, the fatty material, and also any supplementary fat used were added to the meat after dehydration. The batches were stored in enamel kettles at room temperature until packaged. The loaves were packaged in cellophane bags,[3] metal-laminated bags,[4] and in lacquered tin cans [5] under a vacuum of 28 pounds per square inch.

Analytical Procedures: Samples were taken for analysis at various intervals to determine the effects of container, temperature, and incorporation of soya flakes on the niacin, riboflavin, and thiamin contents of the dehydrated pork loaf during storage.

A common extract for the three vitamins analyzed was used. A 10-gm. sample together with 10 gm. of anhydrous sodium sulfate was extracted in a Soxhlet apparatus for at least three hours with petroleum ether [B. P. 35 to 60°C.(95 to 140°F.)] which had been distilled over acid permanganate. This extraction removed fatty material which might cause stimulation in acid production of *Lactobacillus casei* [Strong and Carpenter (1942)], prevented the formation of an emulsion in the extract, and at the same time made possible a total fat analysis to allow calculating the vitamin data to a dry, fat-free basis.

The thoroughly dried, defatted sample (including the sodium sulfate present) was weighed and then the entire sample was ground in a micro-

Wiley mill using a 40-mesh sieve. This gave a very fine powder which provided for uniform sampling and aided extraction.

An eight-gm. sample of the dry, defatted material, containing sodium sulfate with the meat solids, was weighed out and extracted for about 20 minutes at 15 lb. pressure, 121°C.(250°F.), with 60 ml. of 0.5 N HCl. The flask was cooled immediately and 20 ml. of 2.5 M sodium acetate were added to bring the pH to the range 4.0 to 4.5; 160 mg. each of takadiastase and papain were added and the sample was incubated at 37°C. for about 24 hours. The suspension was made to volume, mixed, and filtered through No. 12 Whatman filter paper. The first 10 to 15 ml. of filtrate was discarded The extracts were protected from light throughout this procedure and during subsequent storage to prevent destruction of riboflavin.

The solid:liquid ratios in preparing the extracts as above were satisfactory for the complete extraction of niacin. After the studies were completed it was found that both riboflavin and thiamin were less easily extracted in such concentrated suspensions. If complete extraction had been effected for these two vitamins, the results would be 30 to 50 per cent higher than the figures reported. Accordingly, the values are not absolute but are of value only in so far as they compare with one another.

The method used for the determination of thiamin was the thiochrome method as outlined by the Research Corporation Committee on the Thiochrome Method (1942), with the exception of the extraction procedure used which has been outlined above.

Riboflavin was determined by the microbiological method of Snell and Strong (1939) and modified after the findings of Stokes and Martin (1943) that increased quantities of glucose and sodium acetate resulted in greater acid production. The concentration of glucose and sodium acetate used was two per cent.

The test solution for analysis of riboflavin was derived from the original thiamin extract. Twenty-five ml. of this extract were adjusted to a pH of 6.6 to 6.8 and then diluted to 100 ml. Aliquots of 1.0, 1.5, and 2.0 ml. were taken in duplicate for analysis. Various levels of test solution did not give similar results but instead a "falling off" in riboflavin value was noted with increasing test level. The variation from highest to lowest level was from about 10 to 35 per cent. Such "falling off" effects in the vitamin value have been noted by other workers using *Lactobacillus casei* as the assay organism for riboflavin and pantothenic acid—Strong, Feeney, and Earle (1941); Wegner, Kemmerer, and Fraps (1942); Andrews, Boyd, and Terry (1942); Light and Clarke (1943). Since the nature of this effect is still undetermined, all values at the three assay levels were averaged. It was often noticed that the two higher levels of extract addition were in better agreement than the lowest level, which gave the highest assay value for riboflavin.

The microbiological method of Snell and Wright (1941), as modified by Krehl, Strong, and Elvehjem (1943), was used for the measurement of niacin. The test solution for niacin was derived from the riboflavin dilution. Five-ml. aliquots were made to a volume of 200 ml. and duplicate aliquots of two, three, and four ml. were used for analysis. Checks at the different assay levels of the meat extracts were excellent.

Moisture analysis was accomplished by a five-hour vacuum-oven drying at 95 to 100°C.(203 to 212°F.).

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental data on the effects of temperature, container, and incorporation of soya flakes on the niacin, riboflavin, and thiamin contents of the dehydrated pork loaves during storage are presented (Tables 1, 2, and 3); thiamin retentions are shown graphically (Fig. 1).
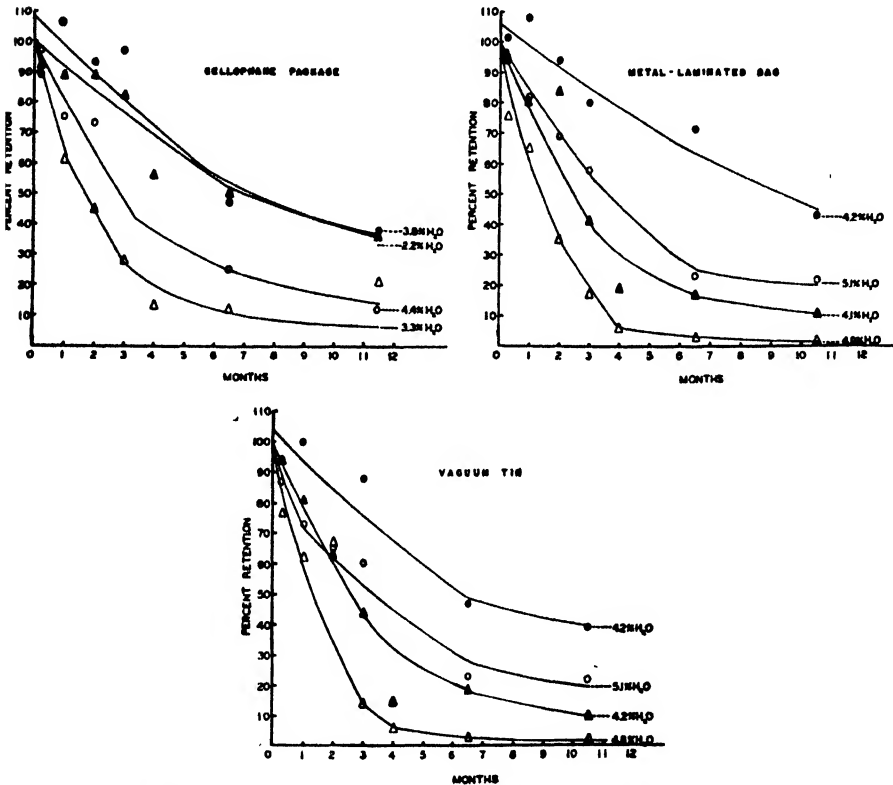
FIG. 1. Effects of temperature, incorporation of soya flakes, container, and moisture content on thiamin retention of dehydrated pork loaves during storage. ○ Control loaf, 70°F.; △ control loaf, 100°F.; ● extended loaf, 70°F.; ▲ extended loaf, 100°F.

In comparing similar lot samplings (that is, one, two, and six and one-half months and the initial, one week, and 10½ months' samplings), it appears that there is at most only slight loss in niacin under the conditions studied (Table 1) when one considers the errors in sampling and in the use of a method which allows for no greater accuracy than approximately 10 per cent. There is some indication that niacin in the extended loaf was affected by storage. Riboflavin was apparently completely unaffected by the conditions of this study (Table 2).

The results obtained on niacin and riboflavin retention are in agreement with the work of the U. S. Department of Agriculture Committee on Dehy-

TABLE 1

Effects of Temperature, Container, and Incorporation of Soya Flakes on Niacin Content [1] of Dehydrated Pork Loaf During Storage

Storage temperature and time	Lot No.	Cellophane package		Metal-laminated bag		Vacuum tin	
		Control	Extended	Control	Extended	Control	Extended
mo.							
Initial	4	210	143	210	143	210	143
70°F.							
1	3	235	117	217	130	172	134
2	3	202	132	194	149	183	186
3	2	......	134	167	110	189	127
6½	3	193	133	182	134 [2]	187	132
10½	4	......	......	191	137	206	......
11½	1	204	135	......	......	......	......
100°F.							
1	3	218	148	223	173	240	164
2	3	199	146	203	171	158	137
3	2	182	121	159	123	161	159
4	2	158 [2]	132 [2]	153 [2]	124 [2]	169 [2]	124 [2]
6½	3	180	128	210	131	199	136
10½	4	......	......	185	123	185	125
11½	1	214	129	......	......	......	......

[1] Expressed as micrograms per gram on a dry, fat-free basis. [2] The fat analysis for these figures was estimated from the average of previous fat determinations on similar samples.

TABLE 2

Effects of Temperature, Container, and Incorporation of Soya Flakes on Riboflavin Content [1] of Dehydrated Pork Loaf During Storage

Storage temperature and time	Lot No.	Cellophane package		Metal-laminated bag		Vacuum tin	
		Control	Extended	Control	Extended	Control	Extended
mo.							
Initial	4	12.2	9.5	12.2	9.5	12.2	9.5
70°F.							
1	3	11.8	8.5	11.7	8.9	10.5	9.1
2	3	12.3	9.2	12.0	9.3	11.5	10.7
3	2	......	11.4	13.4	10.3	13.1	12.0
6½	3	11.7	8.5	11.6	9.1 [2]	12.3	9.1
10½	4	......	......	10.4	8.9	10.9	......
11½	1	9.0	8.2	......	......	......	......
100°F.							
1	3	12.6	8.4	12.9	8.9	13.6	8.7
2	3	12.0	9.4	12.3	11.6	10.1	8.9
3	2	13.1	11.7	13.5	11.7	13.4	13.1
4	2	12.4 [2]	9.6 [2]	11.9 [2]	9.8 [2]	12.7 [2]	10.5 [2]
6½	3	12.5	8.6	14.1	10.0	14.1	9.4
10½	4	......	......	10.3	7.9	11.4	7.4
11½	1	8.3	7.5	......	......	......	......

[1] Expressed as micrograms per gram on a dry, fat-free basis. [2] The fat analysis for these figures was estimated from the average of previous fat determinations on similar samples.

dration of Meat (1944) and of Rice and Robinson (1944), reported above, although their conditions for study were slightly different.

The data on thiamin (Table 3 and Fig. 1), on the other hand, present a different picture from that of the niacin and riboflavin studies. From these data it can be seen that all factors studied in one way or another affect thiamin retention.

TABLE 3

Effects of Temperature, Container, and Incorporation of Soya Flakes on Thiamin Content [1] of Dehydrated Pork Loaf During Storage

Storage temperature and time	Lot No.	Cellophane package		Metal-laminated bag		Vacuum tin	
		Control	Extended	Control	Extended	Control	Extended
mo.							
Initial .	4	19.1	13.7	19.1	13.7	19.1	13.7
70°F.							
1 (wk.)	4	23.2	12.4	22.6	14.1	20.8	13.2
1	3	18.0	14.9	19.8	15.1	17.6	14.0
2	3	17.6	13.0	16.5	13.2	15.7	6.2
3	2	......	13.6	13.9	11.2	14.4	12.3
6½	3	5.9	6.6	5.6	6.3 [2]	5.6	6.6
10½	4	......	......	5.3	6.0	5.2	5.4
11½	1	2.8	5.3	......	......	......	......
100°F.							
1 (wk.)	4	22.0	......	17.9	13.4	18.3	13.1
1	3	14.6	12.4	15.6	11.2	15.0	11.3
2	3	10.7	12.4	8.4	11.7	16.0	8.8
3	2	6.8	11.5	4.0	5.8	3.3	6.2
4	2	3.2 [2]	7.9 [2]	1.4 [2]	2.7 [2]	1.4 [2]	2.1 [2]
6½	3	2.8	7.0	0.8	2.4	0.67	2.7
10½	4	......	......	0.5	1.5	0.28	1.4
11½	1	5.0	5.0	......	......	......	......

[1] Expressed as micrograms per gram on a dry, fat-free basis.   [2] The fat analysis for these figures was estimated from the average of previous fat determinations on similar samples.

The higher the storage temperature the more rapid is the destruction of thiamin. An analysis of the data shows that the higher temperature promotes thiamin destruction at a rate more than twice that found at room temperature in the control loaf. No apparent effect of container is here noted. With the exception of the cellophane-packaged samples, a similar temperature effect is noted in the thiamin content of the soya-extended meat.

Extending the dehydrated pork loaf with soya flakes increases the thiamin retention twofold to threefold.

The effects here noted of temperature and of substituting non-meat ingredients for a part of the dehydrated pork were also observed in the studies of Rice, *et al.* (1943, 1944) and Rice and Robinson (1944). They reported that after 219 days' storage at 26.7°C.(80°F.), the thiamin retention of dehydrated pork is 29 per cent; a thiamin retention of about 25 per cent was found for the control pork loaves in this study stored at room temperature (about 70°F.) for a similar period. At a higher temperature (100°F.) there appears to be very little retention of the thiamin in the

control loaf after about six to seven months. Rice, *et al.* (1943, 1944) studied the effects of incorporating 33 per cent of the non-meat ingredients, in contrast to the 12 per cent of soya flakes (or approximately 19 per cent non-meat ingredients) in this mix prior to dehydration.

An unusual effect of packaging on the quality of the dehydrated meat is observed in the case of thiamin destruction during storage, particularly under elevated temperatures. For the soya-extended, dehydrated pork packaged in cellophane and stored at 100°F., the thiamin content was at all sampling periods very significantly higher than for the same product packaged in either metal-laminated bags or lacquered tins. Thus, after three months, the cellophane-packaged product had lost 18 per cent of the original thiamin, whereas the extended pork packaged in metal-laminated bags and tins showed thiamin losses of 59 and 56 per cent, respectively. After six and a half months at 100°F., the dehydrated meat in cellophane had lost 50 per cent of the thiamin, in contrast to 83 and 81 per cent losses in the other packages. There is some indication that the thiamin in the unextended, dehydrated meat may also be more stable at high temperatures when packaged in cellophane, but the differences are not sufficiently large to be considered significant.

The explanation for the very great differences in thiamin in the extended meat in the several types of packages lies not in a protective effect conferred by cellophane, or a destructive effect from metals, but rather is apparently based on the *moisture content* of the product. Cellophane, in contrast to the other packaging materials studied, is not completely moisture-vapor proof. At the low relative humidity of the 100°F. room (approximately 30 per cent) the dehydrated meat actually lost moisture through the cellophane, the loss being greater from the extended than from the control product. The average moisture contents of the various samples during storage are noted opposite the several curves (Fig. 1). At room temperature, approximately 0.5 per cent moisture was lost through the cellophane in both the control and extended dehydrated meats; at 100°F., the absolute moisture content was lowered by 1.6 to 2.0 per cent.

A possible explanation of the similarity of the thiamine retention at 70 and 100°F. in the extended loaf packaged in cellophane may lie in the differences in moisture contents of these samples. The destructive effect at the higher temperature appears to be balanced by the protective action of a lower moisture content (2.2 per cent in the loaf stored at 100°F. as compared with a moisture content of 3.8 per cent in the loaf stored at 70°F.). Although the moisture contents of the control loaf in cellophane are dissimilar at 70 and 100°F., the difference is not as great as for the extended meat, presumably because the latter is more granular and less fatty, offering a greater surface for evaporation. In the control loaf the ratio of the average moisture content of 70 and 100°F. is 4 to 3 while in the extended loaf, the ratio is 5.2 to 3.

The additional desiccation of the dehydrated pork-and-soya loaf in cellophane at 100°F. serves to delay greatly the hydrolytic destruction of thiamin in the meat. Particularly at low levels of moisture and at high storage temperatures, such as the case of the extended pork at 100°F., a

small change in moisture content effects very striking changes in stability of thiamin in the pork. This fact has obvious implications in both processing and packaging of dehydrated products, and is in line with published data which indicate a prolongation of the shelf life of dehydrated foods when the moisture content is reduced below normal levels.

The data are in agreement with effect of moisture noted by Rice *et al.* (1944). It is apparent that moisture alone does not account for all the variances in thiamin retention. For example, the control loaf packaged in cellophane (70°F.) with an average moisture content of 4.4 per cent at 10½ months' storage has a 16 per cent thiamin retention, while the extended meats stored in the metal-laminated bag and tin can at the same storage time and temperature, and each with a moisture content of 4.2 per cent, have, respectively, 43 and 39 per cent retention. Whatever the role of the extender in promoting thiamin retention, it is certain that it is not evident merely from the absolute moisture content. It was noted that the extended loaf appeared drier and much less greasy than the unextended loaf. This was attributed to the absorption of moisture and fat by the soya flakes. In the absorption of moisture by the soya flakes thiamin destruction in the meat may be prevented. Through the imbibitional forces of such hydrophilic materials as starch and other colloids in the meat extenders, the true moisture content of the dehydrated meat itself may be much lower than is indicated by a gross moisture determination. The lower moisture content in the pork would explain the greater stability of the thiamin.

Storage under a vacuum of 28 pounds per square inch in tin cans apparently makes for no greater thiamin retention than storage in an atmosphere of air.

SUMMARY AND CONCLUSIONS

Studies on the niacin, riboflavin, and thiamin contents of a dehydrated pork loaf stored under varied conditions are reported.

Niacin and riboflavin are shown to be stable for approximately a year at temperatures up to 100°F. This stability is not affected by the container or by replacement of a part of the pork with soya flakes.

Thiamin, on the other hand, is unstable. Elevated storage temperatures exert a definite destructive effect on the thiamin in a dehydrated pork loaf. Data are also presented which indicate the influence of moisture content of the sample upon thiamin retention. Thus, a two-per cent moisture content promotes better thiamin retention than at a level of four per cent.

The container alone does not appear to exert any direct effect on thiamin retention. The cellophane-packaged, soya-extended pork, especially at 100°F., seems particularly stable. This apparent better retention of thiamin in the dehydrated meat packaged in cellophane may be correlated with a lower average moisture content than the average moisture contents of the samples stored in the metal-laminated bag and the vacuum tin.

Thiamin retention in a dehydrated pork product is increased twofold to threefold by substituting soya flakes for 14 per cent of the pork.

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EVALUATION OF SURFACE pH AS A FRESHNESS INDEX FOR FISH FILLETS

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At the present time organoleptic observations are the only generally accepted criteria for judging the freshness of fish. Such tests are very inaccurate, but no accurate objective test has yet been devised which meets the requirements of simplicity, rapidity, low cost, and also does not mutilate the sample and thus render it unfit for sale.

If the pH of fish flesh could be correlated with fish freshness, such a test would come very close to meeting the requirements of an ideal test. pH determinations employing a pH meter are simple, rapid, and accurate, yet the samples are not destroyed. Substitution of pH test papers for the pH meter would increase the simplicity and economy of such a test.

Wood, Sigurdsson, and Dyer (1942) suggested that tests on the *surface* of fish flesh are much more delicate than those on composite samples containing both surface and interior flesh. This is due to the fact that spoilage is much more rapid at the surface than in the interior of fillets. A more recent paper by the same authors, Dyer, Sigurdsson, and Wood (1944), suggests the use of pH on the surface of fillets as an index of freshness. These authors indicate that this is a reliable test even though the pH values of the interior, or of the composite samples, are not sensitive indicators of fish freshness.

The U. S. Army Quartermaster Corps buys fish following an inspection by the Army Veterinary Corps. All tests employed by the Veterinary Corps are organoleptic, and consequently the quality of the fish purchased depends entirely on the judgment of the individual inspector. For this reason the U. S. Army Quartermaster Corps expressed the need for an objective test to replace, or at least to supplement, the organoleptic examination and asked the U. S. Fish and Wildlife Service to investigate the test proposed by Dyer *et al.* (1944). This investigation was then undertaken with the following objects in view:

1. To study the surface pH test to determine whether or not it would be of value to the Army Veterinary Corps inspectors as a freshness yardstick for fish fillet purchases.
2. To simplify it further.
3. To apply the test to the types of fish the U. S. Army generally purchases for mess purposes. These included various species not previously tested by Dyer *et al.* (1944).

The studies here reported were made during the months of September, October, and November, 1944, in laboratory space furnished to the U. S.

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Fish and Wildlife Service by the Gorton-Pew Fisheries Company, Limited, at Gloucester, Massachusetts.

<div align="center">EXPERIMENTAL PROCEDURE</div>

Handling Fish Samples: Fish were received directly from the commercial fishing boats in as fresh a state as possible. Gillnet boats were out for about 10-hour periods; consequently, those fish described as "gillnet"-caught were only six to seven hours out of the water when purchased. Otter trawlers were out for periods of one to two weeks. The fish described as "otter trawl"-caught were obtained from the top of the load wherever possible and, therefore, were still fresh when received. During the storage period on otter-trawl boats, the fish had been iced according to commercial practice.

The fish to be tested (haddock, whiting, dabs, pollock, cod, rosefish, and gray sole) were filleted immediately upon their receipt at the dock. As the fillets from each fish left the filleter's knife, they were paired and wrapped together in a piece of parchment or waxed paper and stored in crushed ice. At all times during the storage period, an excess of ice covered them, so that their temperature was kept constant. At intervals of one or two days, until the fish were completely spoiled, the fillets from six [1] fish were removed from the ice and one fillet from each pair was examined, while its duplicate was wrapped in double parchment or waxed paper and then in plain wrapping paper and placed in a cold room held at —15 to —18°C.(5 to —0.4°F.).

Duplicate fillets were frozen because any test, to be practical for Army use, had to be applicable to fish which had been frozen since most of the fish used by the Army is frozen immediately after filleting and critical inspections are made on the thawed product. These frozen duplicates were held from two weeks to two months at —15 to —18°C. until time was available for their examination. Because the investigation was necessarily of short duration, it was impossible to determine the effect of prolonged frozen storage.

Laboratory Technique: In the laboratory the fillets were tested using a pH meter.[2] Careful checks were made on the meter with commercially prepared buffer solutions at pH 7 at the beginning of each day's work and about every two hours during the examinations.

Preliminary studies indicated that washing electrodes between fillets was unnecessary because the effect of residual films on the glass electrode was overcome by each new contact with the fillet surface. It was found that the effect of fork holes on the pH was not noticeable unless the portion around the hole was very badly discolored and decomposed. The effect of temperature variation on the readings was found to be unimportant for the temperature range in which the fillets were examined, provided the temperature compensator on the pH meter was used.

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[1] Fitzgerald and Conway (1937) found that a minimum of five fish was required to represent the quality of a batch of 2,000 pounds of haddock.

[2] This was a Beckman Model G with external glass (Beckman No. 1190) and calomel (Beckman No. 1170) electrodes.

Preliminary to the examination, the fillets were placed on a table in a room at 20 to 30°C.(68 to 86°F.) and allowed to remain until their temperature had changed from that of melting ice to 10 to 15°C.(50 to 59°F.). Both unfrozen [3] and frozen fish were treated in this manner. Though the frozen fish took three to four hours to thaw, it was believed inadvisable to apply heat or water.

The pH values of the surface and freshly cut interior portions were made at points designated (Fig. 1). The fillet was held firmly against the rigidly supported electrodes, but not firmly enough to puncture the flesh, and a few seconds were allowed for equilibrium to be attained before the reading was taken.
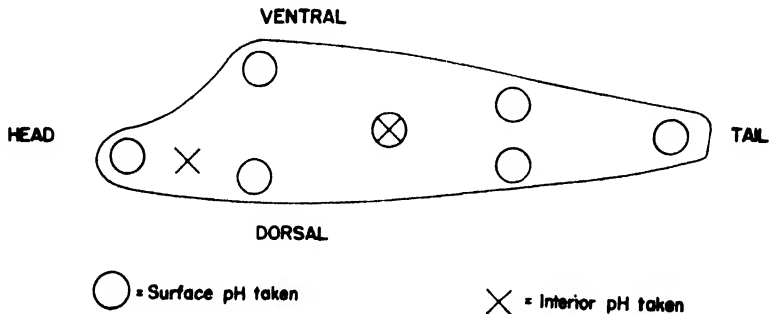
Fig. 1. Positions on fillet at which pH readings were obtained.

The odor of the raw, uncooked fillet was noted when the readings for both the surface and the interior had been obtained. At the beginning of the investigation the opinions of three or four people were averaged, while during the latter part of the study only the author's opinion was considered. Since his opinions had always been in agreement with the average of the other participants, this was believed acceptable.

It may be noted in the accompanying graphs and tables that the organoleptic data were classified as fresh, flat, sweet, stale, and putrid. A fillet designated as "fresh" had the normal odor of freshly caught fish. If it was "flat" there was an absence of odor—normal or otherwise. A "sweet" fillet had an odor not especially unpleasant but reminiscent of watermelon. A "stale" fillet had a characteristic ammonia-like odor (odor of ammonia and other mixed amines) but had not reached the "putrid" stage at which point the odor became obnoxious (hydrogen sulfide, indole, skatole, etc. were present). All fish examined in this study spoiled in the above manner except whiting and rosefish. At the sweet stage whiting developed a perfume-like aroma instead of the usual watermelon-like odor. Rosefish did not become sweet but passed directly from flat to slightly stale.

The author and those who co-operated with him considered the fillets edible through the "sweet" stage, of questionable edibility at "very sweet" and "slightly stale," and inedible at "stale" and at more advanced stages of decomposition. This designation of the exact point at which the fish

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[3] "Unfrozen" is used instead of "fresh" to avoid confusion between the use of the word "fresh" to signify lack of freezing and "fresh" as an organoleptic criterion.

became inedible was determined by judges in the habit of eating fish only of the very freshest nature. Less discriminating people might have placed such a point elsewhere.

INTERPRETATION OF DATA

Accuracy of Methods: A study of values obtained at the various surface sampling points shown (Fig. 1) revealed that the fiducial limits [4] of the seven surface pH readings on individual fillets was, on all samples except those in advanced stages of decomposition, always as small as or smaller than ±0.18. The fiducial limits of the mean [4] were as small as or smaller than ±0.08, except in the most advanced stages of spoilage (Table 1). These values were believed to be sufficiently small for the required accuracy of the test. In the data presented here, all surface pH averages may be assumed to be within ±0.08 pH of the true mean for the fillet described, unless that fillet is "very stale" or worse.

TABLE 1

Precision of pH Determination on Haddock and Gray Sole

| Odor rating | Expected variation among individual surface pH determinations on fillets at various degrees of spoilage [1] | | | | | |
| | Fiducial limits (standard deviation × 2½) | | | Fiducial limits of the mean (standard deviation of mean × 2½) | | |
	Unfrozen otter-trawl haddock	Frozen otter-trawl haddock	Unfrozen otter-trawl gray sole	Unfrozen otter-trawl haddock	Frozen otter-trawl haddock	Unfrozen otter-trawl gray sole
Fresh	.10	.06	.18	.04	.02	.07
Flat	.11	.07	.15	.04	.03	.06
Sl. sweet	.16	......	......	.06	......	......
Sweet	......	......	.18	......	......	.07
Sl. stale	.18	.17	.18	.07	.07	.08
Stale	......	.18	.20	......	.07	.08
Very stale	.19	.39	......	.07	.15	......
Sl. putrid	.70	......	......	.27	......	......
Putrid	......	.27	......	......	.10	......

[1] See Fig. 1 for the positions of the seven surface pH readings taken on each fillet.

It must be understood that organoleptic tests are not always accurate. Had a strictly reliable organoleptic method been available, perhaps better correlation with pH would have been obtained in certain instances. Reference to the graphs accompanying this report reveals that when pH is plotted against organoleptic rating (Figs. 3 to 7), the standard deviation is quite large at times. However, when pH is plotted against storage time (Figs. 3 to 7), the standard deviation is less as a general rule. Thus storage time is indicated to be more dependable than organoleptic rating in these instances.

Theory of pH Changes: Figure 2 shows the ideal curve upon which the surface pH concept as described by Dyer, Sigurdsson, and Wood (1944)

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[4] The "fiducial limits" give the limits within which 95 per cent of the values obtained will be expected to fall. In this case (seven samples), the standard deviation multiplied by 2½ gave the "fiducial limits," and the standard deviation of the mean multiplied by 2½ gave the "fiducial limits of the mean."

is based. The flesh of live fish, such as haddock, has a pH value close to neutrality. According to Benson (1928), however, this is true only of rested muscle; the flesh of fish which have struggled on being caught (gillnet, otter-trawl, and line-caught fish) is slightly acid on death, and it will become more acid as the processes of *rigor mortis* take place. Within four to 24 hours after the death of the fish the pH of the flesh reaches a minimum in the neighborhood of pH 6.5 or lower. In the case of fillets surface and interior pH values are the same at this stage since no surface bacterial action has taken place. For a few days the pH of both the surface and the interior remains in the vicinity of 6.5. The fillets are still considered to be
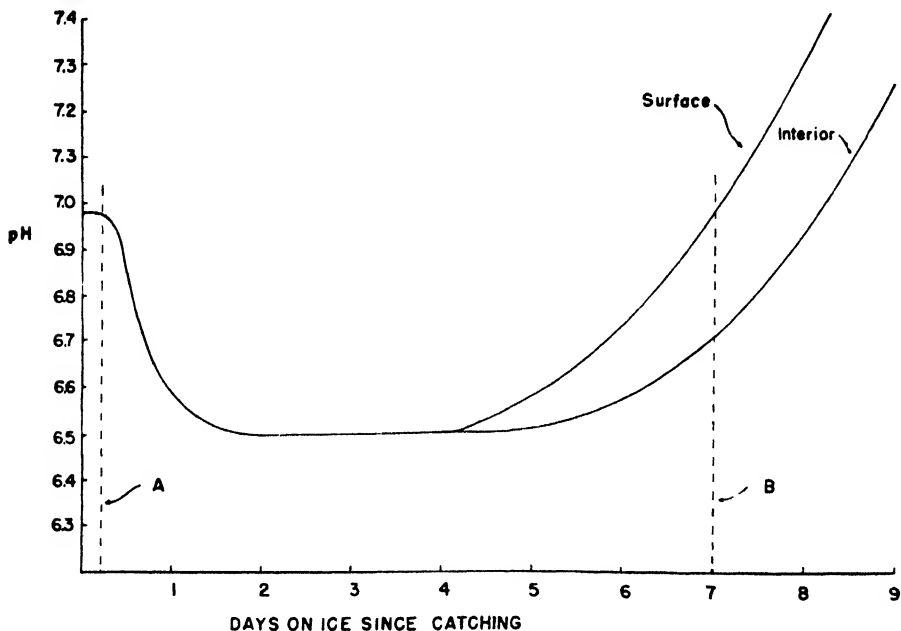
Fig. 2. pH changes occurring during spoilage of such fish as haddock, etc. (This curve is a theoretical ideal and does not represent experimental data.)

in a fresh condition. After a period of time which varies with the temperature, the species of fish, and many other factors, bacterial action at the surface of the fillet releases amines, chiefly trimethylamine, which tend to cause a rise in the pH at the surface. Thus the surface pH increases more rapidly than the interior pH, since the bacteria and/or their metabolic end products filter to the interior only very slowly. This differential has been taken by Dyer *et al.* (1944) as significant in determining the difference between *very* fresh fish and fish at incipient spoilage, even though the surface pH values may be similar. For example, the surface pH immediately on death (Line A, Fig. 2) may be the same as that when spoilage is under way (Line B, Fig. 2), but the interior pH in the latter case would be much lower.

In view of the importance of such pH differences between the surface and interior, points representing fillets whose interior pH values were

close to the average of the seven surface values have been encircled on the graphs accompanying this report, while a cross is used to mark those points representing fillets on which one or more of the interior pH values were significantly lower than this average. If an interior pH departed from the mean of the seven surface values by more than two and a half times the standard deviation of the surface readings of all the *fresh* fillets, the fact was considered significant. As reference to Figs. 3 to 7 will demonstrate, the greater proportion of encircled pH values occurred on fillets in a comparatively fresh condition; thus the contention advanced by Dyer *et al.* (1944) that surface pH rises faster than interior pH is substantiated.
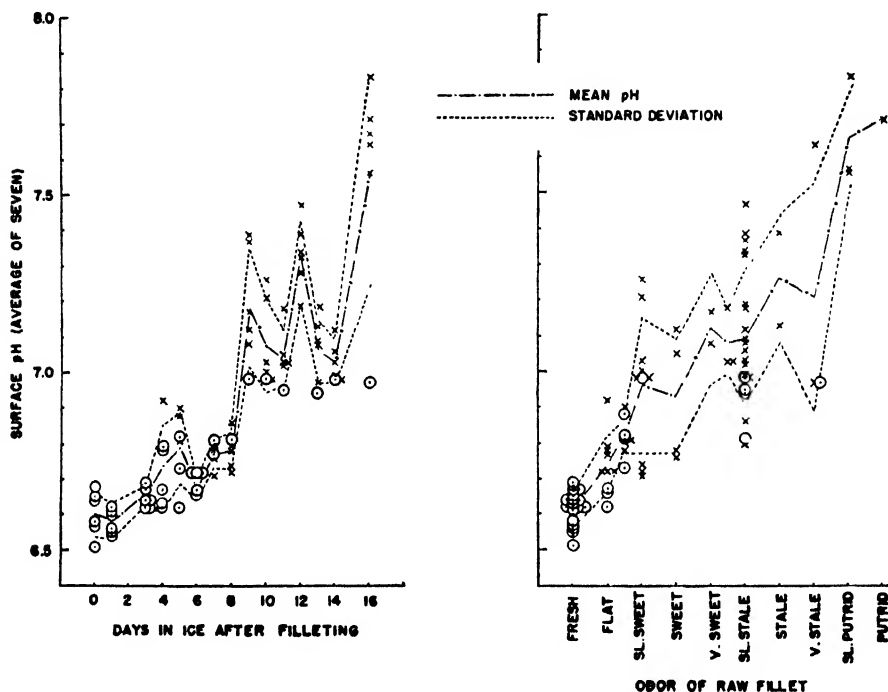
Fig. 3. pH changes during spoilage of otter-trawl haddock (*Melanogrammus aeglefinus*) fillets.[6]

Presentation of Data:[5] Figures 3 to 7 show the relation of surface pH of fillets of various species to storage time in ice after filleting and to organoleptic rating.

For all species studied it was found that the pH rose as spoilage progressed. With some species the dependability of the correlation of pH with the degree of spoilage was good, but with others it was poor.

[6] Because approximately 10,000 pH determinations were made during the course of the investigation, all of this material could not possibly be presented here for lack of space. For this reason, no data are presented for the frozen duplicate fillets, and graphs for some species of fish were deleted because they were very similar to those included with the report.

It is evident (Fig. 3) that pH 6.7 was the dividing line between fresh fillets of haddock and those at incipient spoilage. These results are in agreement with the work of Dyer *et al.* (1944). An experiment run with gillnet-caught haddock showed results very close to those obtained with the otter-trawl haddock, but the limiting line between fresh and spoiling fish was pH 6.8 instead of pH 6.7. This difference might very well have been experimental error.
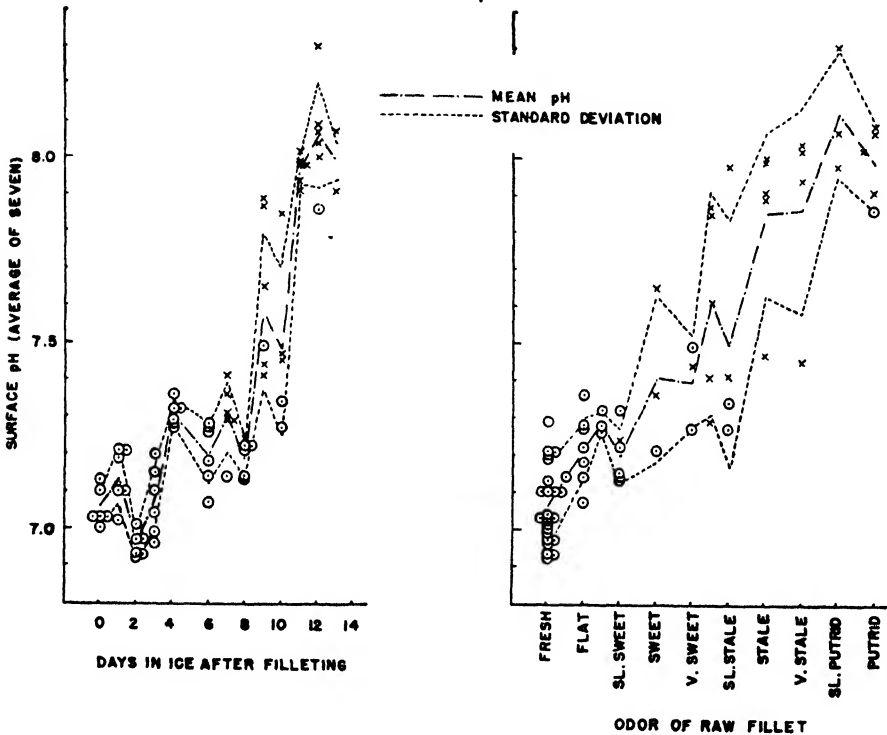
FIG. 4. pH changes during spoilage of whiting (*Merluccius bilinearis*) fillets.[6]

The correlation of pH with freshness of whiting (Fig. 4) was nearly as good as that for haddock. With this species, however, the pH range of the fresh fillets (pH 6.9 to 7.3) was much higher than that for haddock. Dabs (*Hippoglossoides platessoides*) fell into the same category as whiting, though the pH range of fresh samples (pH 6.7 to 7.0) was lower than that for whiting. No data are presented for this species.

With haddock, whiting, and dabs the pH of an individual fillet gave some idea of its condition as evidenced by the consistent rise in pH as spoilage progressed and by the absence of any serious overlap of individual points at different levels. With the rest of the species studied, however, the surface pH values of individual fillets lost their significance and only *average* pH values of several fillets of equal quality could be relied on to give a correlation with storage time or organoleptic rating. In some cases

even this average was very erratic. If more than six fillets had been
examined each day, smoother curves would have resulted.
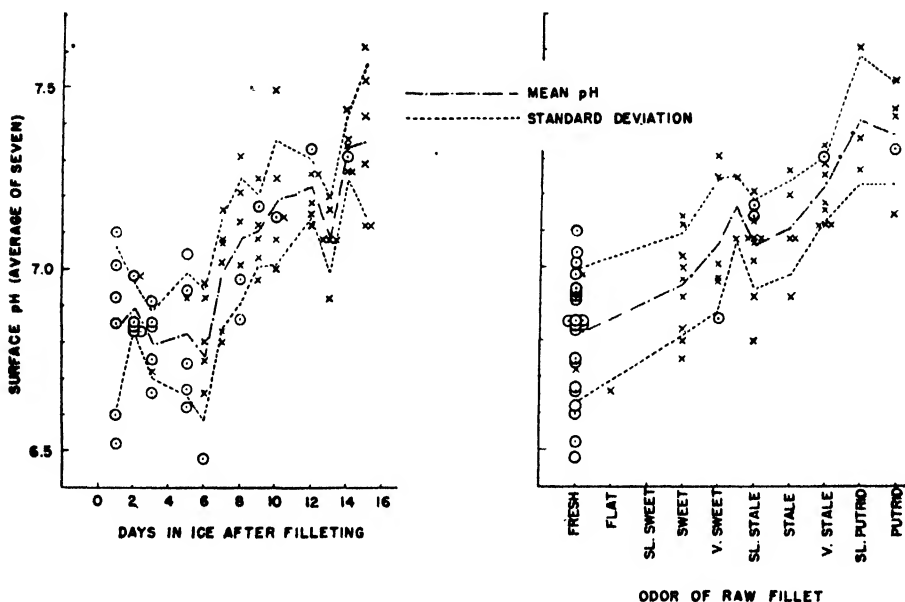
FIG. 5. pH changes during spoilage of gillnet pollock (*Pollachius virens*) fillets.[6]

The data for pollock are presented (Fig. 5); the pH range for the
fresh fillets was very wide (from about 6.5 to 7.1). All but a few of the
fillets whose interior pH values were close to the values at the surface
(as indicated by encircled points) fell in the "fresh" group, and few
were included in this group in which the interior pH was significantly
lower than the surface pH. This would indicate, according to the theory
of the surface concept, that those fresh fillets whose pH values were quite
high might be at the extremely fresh stage (as indicated by Line A, Fig. 2).
This idea was not borne out by the data, however. A comparison of the
data used in plotting the two curves (Fig. 5) showed that pH readings
above 6.9 occurred on fresh fillets on the second, third, and fifth days as
well as on the first day.

Dyer *et al.* (1944) reported that the test worked well for cod. The
data for cod (Fig. 6) show that, in these experiments, the pH values of
individual fillets of a given age or of a given odor rating had little mean-
ing, while the average of these figures showed an upward trend as spoilage
progressed.

The data for gray sole, as presented (Fig. 7), showed that pH may
not be a reliable index of the condition of fillets even though a general
trend upward is easily seen. In this case the surface pH values of indi-
vidual fillets had very little meaning, and even the mean of several of
equal quality was not a wholly dependable criterion. The data for rosefish
(*Sebastes marinus*) are not presented, but the results when plotted look

very much like those of gray sole (Fig. 7); that is, individual points overlapped, the line drawn between mean pH values of fillets of equal quality varied erratically, and the total pH shift was not great.
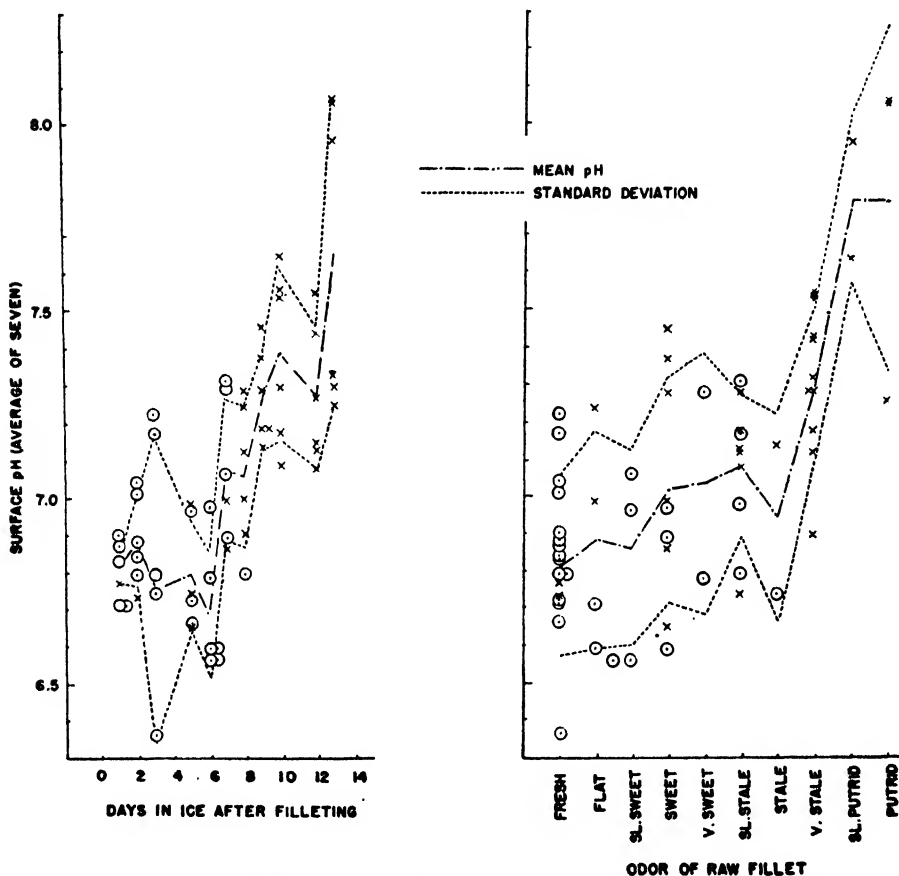
FIG. 6. PH changes during spoilage of gillnet cod (*Gadus callarias*) fillets.[6]

When the data for the thawed duplicate samples were plotted (graphs not presented), it was found that the pH values as a whole dropped considerably as a result of freezing and/or storage in the frozen condition. Upon careful scrutiny of surface pH averages of individual fillets, however, it was found that this tendency for pH to drop on freezing was not at all consistent, and occasional values even rose after such treatment. Because of such irregularity no correction factor can be assigned to a given species. Therefore, since the changes in pH owing to freezing are often very marked, the application of the test to thawed fish as a freshness index seems impractical.

Simplification of the Test: An attempt was made to simplify the test by the use of pH test papers, but none was found in the proper range which was sufficiently accurate for the purpose. This was not surprising in view of similar results reported by other workers, Kolthoff and Rosen-

bloom (1937). It is felt, however, that the use of the pH meter is not a serious drawback to the test.
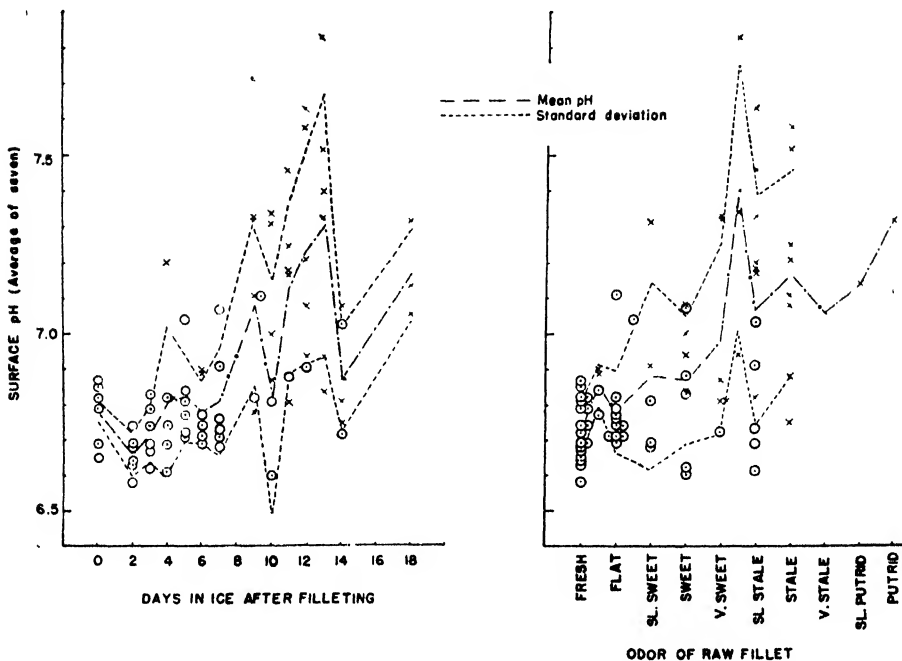
FIG. 7. pH changes during spoilage of otter-trawl gray sole (*Glyptocephalus cynoglossus*) fillets.[6]

DISCUSSION

In considering the application of the test to commercial practice, certain difficulties arise. First, it gives an indication of spoilage which occurs only *after* the filleting process. Undoubtedly, spoilage occurring previous to filleting does have some effect on the test, but surface pH values on new fillets would give no more indication of freshness than those on composite samples or internal flesh, since the filleting operation makes a fresh cut and, therefore, a surface pH taken immediately after filleting would not be a *surface* test but rather an *interior* test. If the test could be applied to a whole or eviscerated fish, this difficulty might be avoided; however, in their reference to its use in this manner, Dyer *et al.* (1944) did not describe how it could be done. The author would expect it to be very difficult to correlate surface pH of whole or eviscerated fish with the freshness of the flesh.

The next limitation, and probably one of equal importance, is the effect of fillet dips on the test. On the Atlantic coast, and to a small extent on the Pacific coast, fillets are dipped in brine after the filleting

[6] In Figs. 3 to 7, encircled points indicate that both interior pH values taken on that fillet were quite close to the surface average, while a cross indicates that at least one interior pH was significantly lower.

process. Occasionally alkaline dips are used to enhance the color and, therefore, the salability of the fillet. If surface pH were used as a measure of freshness, any of the dips used at present might affect the test markedly. Also, when the test became well known, it would be an easy matter for the processor to introduce acid products into the dipping solution of fish of questionable quality and thus lower the pH to correspond to values obtained on a strictly fresh product. Development of the test on whole or eviscerated fish would not circumvent this difficulty since the practice of using carbonate solutions to remove and neutralize malodorous materials from whole fish has been well established in some sections.

As stated previously, the test probably applies only to fish which have never been frozen since, on freezing, erratic pH changes occur which are not connected with freshness in any way.

These limitations have been mentioned to show that, even if it were perfected, the test would not be of practical value to the Army. Furthermore, in commercial application its drawbacks, as outlined above, are not to be disregarded. The test has proved of great value from the standpoint of theoretical interest, however, since it has added to our information on fish spoilage. For laboratory research it will prove very useful.

Before any general application could be made, it would be necessary to determine different limits for each species and to determine the number of fillet examinations that would be required to give a true indication of the condition of a lot of fish. Fitzgerald and Conway (1937) stated that five representative fish indicated the quality of a 2,000-pound lot, but this does not mean that the pH values of five fish represent the quality of that lot, since wide pH variations often occur between fish of equal quality (Figs. 5, 6, and 7). It would also be desirable to determine the effect of (1) variation in types of bacteria present, (2) variation in bacterial load, (3) differences in area of catch, (4) differences in method of catch, and (5) differences in season of the year.

SUMMARY

The surface pH test as described by Dyer, Sigurdsson, and Wood (1944) as a method for determining the freshness of fish fillets was studied, using haddock, whiting, dabs, pollock, cod, rosefish, and gray sole.

As determined by comparison of pH changes with organoleptic grading and with time of storage in ice, the surface pH of fillets tended to rise in all cases as spoilage progressed. The best correlation was obtained with haddock, whiting, and dabs. With the other species examined wide variations occurred in pH among fillets in an equal state of freshness. Thus individual pH values had less significance in the case of these latter species, and only the mean pH of several fillets of equal quality gave a good indication of pH trends on spoilage.

The data obtained on thawed fish did not show a dependable correlation between pH and freshness because of erratic pH changes, probably owing to the freezing and/or storage in the frozen condition.

Even if the pH test were perfected, it would be of limited value because it gives a reliable index of spoilage occurring only after the filleting process and because it would be affected by acid or alkaline dips. These limitations

reduce its value for practical field work but do not preclude its use as a laboratory test in research work.

ACKNOWLEDGMENT

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A STUDY OF YEAST GROWTH-PROMOTING SUBSTANCES
IN WHITE SUGAR [1]

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The white sucrose sugars, beet and cane, are perhaps the nearest approach to chemically pure substances that are included in the daily diet. As a food commodity, sucrose is the cheapest, on a calory basis, on the American market and is used in quantity in more food products than any other prepared ingredient. The value and use of sugar as an ingredient in prepared foods are not limited to its energy-giving content or to its sweetening property. As a preservative in water-containing foods, it has no equal from the standpoint of maintaining flavor, color, turgidity, or keeping quality.

The utility of sugar, like that of many other food ingredients, may be limited by the presence of certain foreign substances. It has been observed that while relatively large amounts of some impurities may be present in a food ingredient without effect when the ingredient is used for a selected food product, only a trace of another impurity may greatly impair the utility of the same ingredient when used in the same product. Sugar is no exception. The presence of bacteria, yeast, and molds in the sugar or of substances which stimulate their growth in sugar solutions is illustrative. For instance, the presence of a large number of viable yeast cells in sugar is of no concern when the sugar is for table use. Such a sugar would not, however, be acceptable for use in the manufacture of beverages. Likewise, the presence of a relatively large amount of yeast growth-promoting substance in sugar for table sweetening would be of no consequence. Yet such a sugar would not be desirable for use in the manufacture of beverages or similar products which might become contaminated with yeasts through either the sugar itself or from other sources. Thus, because of the extensive use of sugar in beverages, fountain sirups, flavoring extracts, and sweetened condensed milk, which are subject to spoilage by yeast, the presence

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of yeast growth-promoting substances in sugar is a problem of vital importance among the manufacturers as well as the users of sugar.

LITERATURE REVIEW

Sucrose has been used as a constituent of synthetic nutrient solutions for the propagation of yeasts since Pasteur (1860) first reported the results of his classic experiments on yeast growth in mineral-salt-sugar solutions. Windisch (1902), Amand (1902), and Kossowicz (1903) were among the first workers to observe the growth-promoting effects of sugar on the growth of yeasts, and although the growth-promoting substances were not identified, it was generally agreed by most workers that they were organic in nature. These substances, like "bios," which was determined by Wildiers (1901) to be indispensable for the fullest development of yeast in synthetic nutrient solutions, were known to be present in trace quantities. It remained for Funk and Freedman (1923) to be the first to recognize the presence of a yeast growth-promoting vitamin in cane sugar as a result of their attempts to cultivate yeasts in synthetic nutrient solutions for animal-feeding tests. Subsequent studies have revealed the complex nature of the yeast growth-promoting substances. These have been extensively reviewed by several workers, including Tanner (1925), Miller (1930), and Peskett (1933), each of whom included extensive bibliographies in his review. More recently, Williams (1941) reviewed the specific yeast nutrilites in consideration of the most recent knowledge available on the subject. Among the group of known substances which influence the growth of yeast is biotin, a member of the vitamin B complex, first reported to be present in sugar by Cheldelin and Williams (1942). These workers report the presence of less than 0.004 microgram per gram but do not state the type of sugar examined.

EXPERIMENTAL PROCEDURE

Most previous workers have been concerned with the effect of growth-promoting substances and impurities in sugar from the standpoint of yeast nutrition. Hall, James, and Stuart (1933) were the first to consider the subject from the standpoint of commercial manufacture and use of sugar, when it became apparent that there were differences between the biological quality of sugars of the same type, i.e., beet sugar and cane sugar, and that these differences were noticeable in their use in food products, especially beverages.

In the previous study it was observed that when a definite number of cells of a culture of *Saccharomyces cerevisiae,* isolated from a bottle of carbonated beverage, were inoculated into sterilized 10-per cent solutions of beet or cane sugar, the size of the inoculum varied from no increase to manyfold after 72 hours' incubation. There was no multiplication of cells in a C. P. grade sucrose. This indicated that a yeast growth-promoting substance was present as an impurity in variable amounts in sugar. As a study was already being made annually of the chemical impurities of campaign-composite samples of beet sugar manufactured by most domestic factories, it was desired to use a rapid biological method for determining the relative amount of growth-promoting substances in the same samples

for correlation with the amount of impurities as determined by chemical analysis. It was desired also to identify the growth-promoting substances and to study the possibility of their elimination from sugar during the process of its manufacture.

The method used for determining the relative amount of growth-promoting substances in sugar throughout this study was described in an earlier paper by Hall, James, and Stuart (1933). Each sample of sugar in a group was similarly prepared in 10-per cent solution, sterilized, and inoculated with approximately 50,000 yeast cells per milliliter. After 72 hours' incubation at 30°C.(86°F.) the ratio of the number of cells per milliliter of the resulting crop to the number per milliliter of the inoculated solution was given as the value for the yeast-inoculum multiple. The range of values, determined each year on 29 to 87 factory samples from 1931 to 1942, is shown (Table 1).

TABLE 1

Average, Minimum, and Maximum Yeast-Inoculum Multiples for
Beet-Sugar Samples for 1931 to 1942

Year	Number of samples	Number of cells inoculated per ml.	Yeast-inoculum multiples		
			Average	Minimum	Maximum
1931	29	35,000	6.6	1.0	23.0
1932	68	47,000	8.5	1.0	80.8 [1]
1933	58	50,000	6.9	1.0	30.4
1934	59	48,000	4.8	1.0	13.4
1935	65	49,000	12.4	1.0	59.6
1936	70	47,000	8.6	1.1	27.4
1937	70	51,000	8.0	2.6	19.2
1938	77	50,000	7.6	1.4	25.2 [2]
1939	75	53,400	9.1	1.9	40.2 [3]
1940	87	52,000	8.0	1.1	18.4
1941	87	50,000	6.6	1.0	16.6
1942	87	51,000	4.2	1.0	18.8

[1] Only one sample above 39.2.   [2] Only one sample above 18.2.   [3] Only one sample above 24.2.

The average yearly yeast inoculum-multiple values show a marked variation over the 12-year period. Beginning with the campaign year 1932, there are three distinct periods during which the average multiple values showed yearly decreases, only to be followed by sudden increases which are again followed by yearly decreases. It would appear that these trends would be of value for correlation with factory-operation data or with chemical analysis of impurities of the same samples, but no other such trends were noted. A possible explanation for the failure to obtain correlation with other available data is that the stimulative substances are present in amounts too small to be quantitatively associated with other nonsugar substances. It is significant that the maximum multiple values for these sugars, with but two or three exceptions, do not exceed values that are considered to be a safe maximum limit for use of the sugar in liquid food products, such as beverages, when initially contaminated with less than 50,000 yeast cells per milliliter of beverage, provided that stimulants are not supplied from other ingredients.

Nearly every year, as the study progressed, the number of operating factories increased mainly as a result of resumption of operation of previously idle factories or of older factories moving to new locations. Numerous improvements made during this period of time in processing equipment and procedures greatly increased operation efficiencies, resulting in an improvement in the general quality of the sugar from nearly every factory. These changes are reflected by decreased amounts of non-sugar substances, for example, the continual reduction of ash content of the sugar. A correlation, although not exact, exists between the amount of ash and the relative amount of yeast growth-promoting substance in the samples. This relation is apparent only when the samples are divided into two groups, i.e., those below and those above the average yeast inoculum-multiple value, and when the average ash content is obtained for all the samples in each group. On this basis, the average ash content is lower for the group of samples having the lower average yeast inoculum-multiple value. Likewise, the average ash content is higher for the group of samples having the higher average yeast inoculum-multiple value; the results that show this relationship are given (Table 2).

TABLE 2

Relationship Between Yeast-Inoculum Multiples and Ash Content of Composite Beet-Sugar Samples for 1931 to 1942

Year	Average for all samples		Ash average for group		Number of samples in group	
	Yeast-inoculum multiple	Ash [1]	With yeast-inoculum multiple below average	With yeast-inoculum multiple above average	With yeast-inoculum multiple below average	With yeast-inoculum multiple above average
		pct.	pct.	pct.		
1931	6.6	.0274	.0244	.0348	19	10
1932	8.5	.0245	.0219	.0246	50	18
1933	6.9	.0197	.0186	.0222	40	18
1934	4.8	.0140	.0108	.0169	36	23
1935	12.4	.0132	.0116	.0156	47	18
1936	8.6	.0135	.0130	.0151	38	32
1937	8.0	.0116	.0105	.0151	37	33
1938	7.6	.0109	.0106	.0116	47	30
1939	9.1	.0110	.0104	.0117	37	38
1940	8.0	.0099	.0095	.0104	45	42
1941	8.6	.0091	.0082	.0101	49	38
1942	4.2	.0111	.0140	.0119	53	34

[1] The ash determinations were made by Sam Byall, Chemist, Bureau of Agricultural and Industrial Chemistry, as part of a study of sugar impurities.

The relationship in samples for each year between yeast-inoculum multiple and the ash content, when samples are grouped according to whether their ash contents are below or above the average, indicates that the amount of growth-promoting substance is directly related to the ash content of the sugar. These results would indicate that stimulation might result from the ash, but this is not so because the ash of sugar does not exert a stimulative effect on the yeast.

The relationship between the ash content and the yeast-inoculum multiple for the campaign-composite samples examined yearly was studied on

the basis of individual samples over the 12-year period with the hope that a direct correlation might be found between these values which would permit estimation of the amount of growth-promoting substances from the ash content of the samples. If it were possible to use the ash content of the sugar, which is usually estimated daily during its manufacture, as a basis for estimating the relative amount of growth-promoting substances, information would become available immediately regarding this quality of the sugar. To study this possibility the yeast inoculum-multiple value and the ash content of campaign-composite samples from individual factories were tabulated on a yearly basis, from 1931 through 1941; typical data are given (Table 3).

As it was immediately evident that there was no exact correlation between the ash content and the relative amount of growth-promoting substance in the sugar, the tabulated data were limited to samples obtained from 12 factories. Samples A to F and G to L were from Steffen and non-Steffen factories, respectively, which were distributed throughout the entire beet sugar-producing area.

It is readily seen from these data that there is no relationship between the yearly yeast inoculum-multiple value and the ash content of the sugar from individual factories. There is, with only a few exceptions, a gradual yearly reduction in the ash content of most samples over the 12-year period. The reduction for the individual samples corresponds very closely with that obtained for the yearly averages for all composite samples. There is no such reduction or trend shown throughout the period for the yeast inoculum-multiple values to correspond with the ash values. With the exception of samples from Factories C, F, I, and L, there is a downward trend in both values for the years 1932, 1933, and 1934. Inasmuch as the values of the yeast-inoculum multiples were so irregular in comparison with the ash content for the remaining years, these results did not appear to be particularly significant. It was concluded, therefore, that the ash content of the sugar cannot be used as a measure of the amount of yeast growth-promoting substances present. There are two possible explanations for this: (1) the growth-promoting substances are organic and are consequently not a part of the ash, and (2) the amount of stimulating substances is too small to be quantitatively correlated with other impurities, at least when estimated by this method.

YEAST GROWTH IN SUGAR AS A MEASURE OF UNIFORM FACTORY OPERATION

Samples of sugar were obtained from individual consecutive strikes from three factories in order to determine the relative amount of growth-promoting substances in consecutive strikes. It was desired to obtain information regarding the uniformity of operation of the factories by determining the value of the mean deviation for the samples from each. Sixty-six samples were obtained. The yeast inoculum-multiple value was determined for each individual sample, and the average (mean) and mean deviation values were calculated; results are given (Table 4).

The average yeast inoculum-multiple values for the samples from Factories A, B, and C are 9.9, 5.8, and 4.7, respectively. Corresponding to

TABLE 3

Relationship Between Yeast-Inoculum Multiples and Ash Content of Composite Beet-Sugar Samples From Individual Factories

Factory		1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941
A[1]	Multiple	X[4]	10.9	6.8	2.8	11.4	4.7	5.4	7.8	8.5	3.0	5.0
	Ash[3]	X	.0286	.0138	.0048	.0098	.0085	.0080	.0070	.0076	.0082	.0083
B	Multiple	X	7.2	4.7	2.6	2.8	4.0	7.2	6.6	5.4	10.9	10.0
	Ash	X	.0206	.0140	.0095	.0093	.0108	.0110	.0085	.0085	.0104	.0095
C	Multiple	X	11.8	14.1	7.8	23.2	11.0	9.6	14.2	12.0	9.4	6.4
	Ash	X	.0318	.0318	.0205	.0138	.0182	.0175	.0100	.0147	.0163	.0132
D	Multiple	11.1	5.2	4.1	3.6	5.3	3.7	8.2	6.4	3.0	1.1	5.0
	Ash	.0160	.0196	.0160	.0065	.0058	.0106	.0055	.0070	.0069	.0068	.0083
E	Multiple	8.4	4.6	4.7	3.0	11.0	6.0	9.8	3.4	2.9	6.7	6.6
	Ash	.0180	.0098	.0085	.0100	.0110	.0085	.0215	.0108	.0085	.0078	.0078
F	Multiple	1.4	3.4	4.4	8.4	3.9	10.4	2.8	5.6	8.1	10.9	4.2
	Ash	.0120	.0102	.0115	.0080	.0060	.0057	.0045	.0070	.0059	.0047	.0062
G[2]	Multiple	X	12.0	3.3	1.2	4.2	4.4	9.2	7.6	12.6	12.5	5.6
	Ash	X	.0244	.0070	.0075	.0100	.0095	.0090	.0051	.0072	.0069	.0074
H	Multiple	X	3.8	1.2	1.0	8.9	3.8	9.4	4.8	4.4	10.9	2.2
	Ash	X	.0214	.0053	.0040	.0118	.0076	.0070	.0070	.0092	.0084	.0102
I	Multiple	2.4	3.0	5.7	1.6	3.3	19.1	2.6	4.6	14.3	6.6	5.4
	Ash	.0170	.0180	.0145	.0070	.0090	.0120	.0085	.0070	.0076	.0071	.0088
J	Multiple	1.0	5.4	3.3	X	X	7.5	6.0	5.8	8.5	10.5	X
	Ash	.0150	.0156	.0235	X	X	.0185	.0135	.0160	.0139	.0147	X
K	Multiple	20.1	8.8	6.3	3.2	33.2	10.0	9.2	7.6	7.3	X	X
	Ash	.0170	.0193	.0173	.0120	.0145	.0102	.0110	.0110	.0108	X	X
L	Multiple	1.0	3.2	5.0	1.2	9.2	2.7	6.6	4.2	12.4	1.5	3.6
	Ash	.0210	.0197	.0170	.0100	.0085	.0121	.0090	.0095	.0084	.0082	.0068
Average on factories	Multiple	6.6	8.5	6.9	4.8	12.4	8.6	8.0	7.6	9.1	8.0	6.6
	Ash	.0274	.0245	.0197	.0140	.0132	.0135	.0116	.0109	.0110	.0099	.0091

[1] A to F are Steffen factories. [2] G to L are non-Steffen factories. [3] Per cent. [4] X indicates no sample was received or the factory did not operate.

these values are the mean deviation values of 2.4, 1.0, and 0.53, respectively. By using these values as a numerical index of the uniformity of operation, and the value zero for perfect operation, Factory C is seen to have the best operation. The operations of Factories B and A are increasingly less uniform in that order. There were no known irregularities in the operation of Factories A and B to account for the nonuniformity of yeast inoculum-multiple values.

TABLE 4

Relative Amount of Growth-Promoting Substances in Consecutive Strikes of Beet Sugar

Factory A		Factory B		Factory C	
Strike No.	Yeast-inoculum multiple	Strike No.	Yeast-inoculum multiple	Strike No.	Yeast-inoculum multiple
220	8.3	394	5.8	184	4.2
221	6.4	395	6.2	185	3.8
222	8.0	396	6.9	186	4.3
223	10.1	397	5.8	187	5.8
224	8.1	398	3.2	188	5.1
229	12.9	399	5.6	189	4.3
241	11.6	400	8.3	190	5.0
242	9.3	401	5.6	191	4.4
244	15.2	402	6.4	192	5.4
245	15.9	403	3.2	193	5.4
246	11.0	404	4.1	194	5.8
247	6.0	405	4.9	195	5.2
248	5.6	406	4.8	196	5.8
249	9.3	407	8.0	197	4.7
250	11.5	408	4.1	198	4.8
......	......	409	7.1	199	3.6
......	......	410	6.0	200	3.4
......	......	411	5.2	201	4.4
......	......	412	6.3	202	4.9
......	......	413	5.1	203	4.3
......	......	414	7.1	204	4.9
......	......	415	6.4	205	4.6
......	......	416	7.3	206	5.0
......	......	417	5.8	207	4.4
......	......	418	7.5	208	5.4
Average (mean)........9.9		Average (mean)........5.8		Average (mean)........4.7	
Mean deviation 2.4		Mean deviation 1.0		Mean deviation 0.53	

IDENTIFICATION OF BIOTIN AS A STIMULANT IN SUGAR

A number of alcohol-free extracts were prepared from beet-sugar and cane-sugar samples by recrystallization of individual samples and were assayed[5] for biotin, pantothenic acid, para-amino benzoic acid, and folic acid. The assays were made on alcohol-free extracts prepared by recrystallization of individual samples from 80-per cent alcohol, a method shown by Hall and James (1936) to be effective in the removal of yeast growth-promoting substances from crystalline sugar. The percentage of sugar solids remaining in the extract was determined, and the amount of biotin

was calculated on the basis of the original dry sugar. The assay was carried out by the method (unpublished) of Daniel and Kline, a microbiological procedure employing *Lactobacillus arabinosis* with a purified culture medium. Although the number of samples examined was insufficient to permit drawing definite conclusions, the results given (Table 5) indicate a possible relationship between the amount of biotin present and the yeast growth-promoting ability of the sugar as measured by the yeast inoculum-multiple values.

TABLE 5

Relationship Between Amount of Biotin and Yeast-Inoculum Multiples in Beet and Cane Sugar

Sample No.	Type of sugar	Biotin (μmg. per gram)	Yeast-inoculum multiple
C-1	Cane	.445	7.8
C-4	Cane	.390	7.2
C-3	Cane	.075	6.1
4166	Beet	.060	3.7
C-5	Cane	.055	14.5
4165	Beet	.040	5.5
C-2	Cane	.035	3.2
4111	Beet	.025	2.9

Biotin was found to be present in all samples assayed, but pantothenic acid, para-amino benzoic acid, and folic acid were not detected. The amount varied from 0.025 to 0.445 micromilligram per gram of sugar. By arranging the samples in descending order of the amount of biotin present, it is seen that the yeast-inoculum multiples, except for Samples 4166 and C-5, are likewise in descending order. Although the reason for the irregular results obtained for these two samples is not known, there is a possibility that other undetermined growth-promoting substances are present in the second one. Existing conditions did not permit continuation of the investigation of this subject.

The biotin requirements of various yeasts have been studied by several workers. Kögl and Fries (1937) showed that only 0.00004 microgram of biotin is necessary in two ml. of culture medium to cause a 100-per cent increase in the crop of Rasse M. yeast in five hours. Snell, Eakin, and Williams (1940) believe that biotin is the limiting factor in the growth of yeast and showed that the presence of a minute amount of pyridoxin greatly increased its growth-promoting effect. They also believe that yeast is able to synthesize biotin during growth. Lochhead and Landerkin (1942) found biotin to be essential to the growth of all 23 strains of osmophilic yeasts studied by them.

RELATIONSHIP BETWEEN STIMULANT IN INTERMEDIATE PRODUCTS AND SUGAR

The reason for the failure of some sugars to show growth-promoting activity, while others showed widespread variations, was investigated from the standpoint of the manufacturing process in the hope that means would be found for eliminating the stimulant from all sugars. A number

of intermediate products, including juice, sirup, and massecuites obtained from several factories, were studied for their ability to stimulate the growth of yeast cells. It was found that all the products stimulated the growth of yeast in solution, the extent of growth varying with products from different factories. The stimulative substances are believed to originate in the sugar beet and are not destroyed during the sugar-recovery process.

Since the most extensive multiplication of the yeast occurred in the white-sugar massecuites, which is the most concentrated form of sugar and sirup obtained in the process, it is evident that there is a substantial concentration of the stimulant in this product. The study of the relative amount of growth-promoting substances in white sugar versus massecuites was extended to include campaign-composite samples of each from 14 Steffen factories and 13 non-Steffen factories. Each product was made up in 10-per cent solution of sugar, and its yeast-inoculum multiple was determined; results are given (Table 6).

TABLE 6

Comparison of Relative Amounts of Growth-Promoting Substances in Massecuites and Sugar From Steffen and Non-Steffen Factories

Steffen Yeast-inoculum multiple			Non-Steffen Yeast-inoculum multiple		
Factory	Massecuites	Sugar	Factory	Massecuites	Sugar
A............................	92	10.0	A............................	168	8.6
B............................	154	10.6	B............................	272	8.6
C............................	156	8.0	C............................	322	5.6
D............................	162	9.8	D............................	326	6.6
E............................	180	6.6	E............................	354	10.4
F............................	198	4.2	F............................	356	4.8
G............................	214	16.6	G............................	360	4.8
H............................	230	3.8	H............................	411	4.4
I............................	232	6.3	I............................	452	10.2
J............................	260	9.0	J............................	454	3.2
K............................	330	3.6	K............................	526	3.6
L............................	332	8.0	L............................	660	11.4
M............................	378	5.0	M............................	842	6.8
N............................	414	12.2	....	......	......

There was no uniform relationship between the relative amounts of stimulants in the sugar and massecuites (Table 6). This was illustrated in the disparity which existed between Samples A and H from the Steffen factories. The sugar from Factory A had a yeast inoculum-multiple value of 10 as compared with 3.8 for that from Factory H; yet the massecuites from Factory H had a yeast inoculum-multiple value of 230 as compared with 92 for that from Factory A. Similar relationships also exist between the samples obtained from the non-Steffen factories. The failure to eliminate the stimulant from the sugar while working the massecuites can only result in its presence in the sugar. Too much emphasis cannot be placed on the elimination from the sugar of all nonsugar substances while working the massecuites.

LOCATION OF GROWTH-PROMOTING SUBSTANCES WITH RESPECT TO CRYSTAL
SURFACES AND INFLUENCE OF QUANTITY OF CENTRIFUGAL WASH WATER

In a previous paper by Hall and James (1936) it was shown that the
greater part of the growth-promoting substances in sugar was located at
or near the crystal surfaces. The average reduction of the yeast-inoculum
multiple was more than 50 per cent in 12 samples of sugar when an average
of 8.4 per cent of the crystal was removed by mingling in a nearly satu-
rated sirup made from sugar which did not promote the growth of yeast.

The distribution of these growth-promoting substances is somewhat
similar to the distribution of other nonsugar impurities in sugar. Paine
and Balch (1926) found that the colloidal matter present was fairly uni-
formly distributed throughout the crystal. Keane, Ambler, and Byall
(1935) showed by mingling crystals of uniform size with sugar solutions
in which from 4.3 to 30 per cent of the crystals were dissolved away that
over 50 per cent of the ash, sulfates, chlorides, sodium, potassium, and
total nitrogen was located in the outer five per cent of the crystal; whereas
color, calcium, and sulfites were more uniformly distributed throughout
the whole crystal.

In order to determine if greater elimination of growth-promoting sub-
stances could be obtained from crystal surfaces during centrifugation by
increased quantities of centrifugal wash water, the quantity of water was
changed in successive baskets of massecuites while spinning a strike of
white sugar. One machine was selected in each of four factories, and for
four successive baskets the quantity of water normally used was increased
or decreased by two quarts. After each change, a sample of finished sugar
was collected from the machine while the sugar was being plowed out, so
that the sample was representative of the entire basket. The yeast-inoculum
multiple of each sample was determined. The results, together with the
quantity and temperature of water used, are given (Table 7).

TABLE 7
*Influence on Yeast Inoculum-Multiple Value of Quantity of
Centrifugal Wash Water*

Factory A		Factory B	
Quarts of water at 88°C. (190.4°F.)	Yeast-inoculum multiple	Quarts of water at 72°C. (161.6°F.)	Yeast-inoculum multiple
14 [1]	22.7	16 [1]	13.4
16	20.5	18	9.6
18	19.4	20	7.4
20	14.8	22	3.6
Factory C		Factory D	
Quarts of water at 86°C. (186.8°F.)	Yeast-inoculum multiple	Quarts of water at 86°C. (186.8°F.)	Yeast-inoculum multiple
15	8.8	24 [1]	3.8
17	8.8	22	3.4
19	6.4	20	6.0
21	4.8	18	7.8

[1] Quarts normally used.

There was a decrease in the yeast inoculum-multiple value of samples from Factories A, B, and C (except one change in sample from Factory C) when the normal quantity of wash water was increased by two quarts per machine for successive baskets of sugar. Conversely, when the normal quantity of water was decreased by two quarts per basket with sample from Factory D, the yeast inoculum-multiple value increased (except for one change). These results give further evidence that the yeast growth-promoting substance is located at or near the surface of the crystal and that it can be effectively diminished by controlled quantities of additional wash water.

SUMMARY

A study of the yeast growth-promoting properties of beet and cane sugar has shown that varying amounts of substances are present in different sugars. A substance identified as biotin was found to be present in amounts from 0.025 to 0.445 micromilligram per gram of sugar. Although there is no exact correlation of the amount of growth-promoting substances with other known impurities of the sugar, there is a correlation of averaged yeast inoculum-multiple values with averaged ash values. The growth-promoting substances, like most other nonsugar impurities, are located at or near the surface of sugar crystals and can be effectively diminished by controlled quantities of additional wash water while the massecuites are being centrifuged.

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REHYDRATION OF DEHYDRATED MEAT [1]

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The belief is generally held among those who have knowledge of dehydrated meat that, other factors being equal, the greater the capacity of a particular lot of the product to absorb and retain moisture the closer its approach to fresh meat with respect to certain elements of palatability. In fact, that such is the case appears to be a logical and safe assumption. Inclusion of studies of the possible meat qualities and processing factors affecting rehydration was therefore regarded as essential in the emergency meat-dehydration investigations.

OBJECTIVES

The purpose of the studies was to throw light on the effects of protein or fat content, size of particle, method of pretreatment, and temperature of drying on the capacity of the finished product to take up moisture. It was believed the results would aid in evaluating processing methods.

EXPERIMENTAL PROCEDURE

No suitable method for determining the rehydration capacity of dehydrated meat appeared to be available. Consequently it was necessary to devise such a method. That the method finally adopted for the work should be sufficiently exact to yield readily duplicated results and to measure small differences between samples was regarded as essential.

In planning the preliminary study on method a certain soil centrifuge that was available appeared promising for the purpose. It was equipped with a circular metal basket having perforations in the side which allowed moisture to escape. Into the basket were fitted 16 square metal boxes with lids and wire-screen bottoms. The distance between the bottom of each sample box and the center of the revolving head of the centrifuge was six inches.

The weighed sample of meat in the box was soaked in water for a given time, centrifuged to remove excess water, and reweighed to determine the weight of water retained. Tests involved soaking samples for 15 minutes to two hours and showed that soaking for one hour was adequate. In order to subject the material to a centrifugal force just sufficient to eliminate surface water, but not to force out that absorbed by the meat tissue, different periods of time and different centrifuge speeds were tried. Centrifuging for 40 minutes at 1,500 r.p.m. was found satisfactory since good

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checks were obtained between duplicate samples in successive tests as well as in the same run.

The procedure finally adopted consisted in weighing a 10-gram sample from a lot of dehydrated meat into a centrifuge box of known weight, fitted with a filter-paper lining. The sample in the box was soaked for one hour in distilled water at a temperature of 28 to 30°C. (82.4 to 86°F.), drained for a few minutes, and centrifuged for a total of 40 minutes at 1,500 r.p.m. Duplicate samples were balanced opposite each other in the centrifuge. Approximately five minutes were required to increase the speed of the centrifuge to 1,500 r.p.m. after the current was turned on, and no brake was applied when the current was turned off at the end of 40 minutes. The box containing the meat was then weighed, and the weight of water retained by the 10-gram sample was calculated. An allowance of 0.3 gram was made for water absorbed by the filter-paper lining. Results were expressed as grams of water retained per 100 grams of meat and were the mean of from three to seven determinations on each sample. The deviation from the mean in most cases did not exceed ± three grams.

RESULTS

It is logical to believe that in the rehydration of dehydrated meat the water absorption is done largely by the protein tissues and to a negligible extent by the fat. Moreover, since the moisture content of different lots of dehydrated meat should be fairly constant, at 10 per cent or below, the higher the protein content the lower the fat content becomes and vice versa. The protein content, fat content, or the protein-fat ratio would appear to be a factor of much significance in relation to rehydration capacity.

In a group of seven lots of dehydrated pork, all processed alike in vacuum-rotary Drier A,[3] the protein content ranged from 64.8 to 55.5 per cent with the fat content ranging from 26.5 to 39.4 per cent. Rehydration values varied from 38.5 grams for the sample lowest in fat to 16.6 grams for the highest in fat. The corresponding protein-fat ratios were 2.34:1 and 1.41:1.

When fat was removed by suction in increasing amounts from a lot of high-fat dehydrated pork produced by the vacuum-rotary method, thus widening the protein-fat ratio, rehydration values of five resulting sub-samples increased from 7.4 grams on one extreme to 37.3 grams on the other. This occurred despite the fact that in consequence of removing fat the proportion of moisture in the dehydrated product had some tendency to increase, with the last subsample containing appreciably more moisture than the first.

Owing to the cohesion of some meat particles and the disintegration of others during the processing, as well as other factors, the use of a grinder plate with holes of a given diameter is no assurance that the particles of a particular lot of dried product will be uniform in size.

When two samples of dehydrated pork of approximately the same protein-fat ratio and processing treatment, except that one was ground

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[3] Described in the first article in this series which appeared in *Food Research* 10, No. 5, 379-391.

TABLE 1

Influence of Composition and Particle Size on Rehydration Capacity of Drum-Precooked, Air-Dried Pork

Type of meat	Sample No.	Precooking conditions			Drying conditions			Composition of dehydrated meat			Rehydration value (grams water per 100 grams meat)
		Steam pressure	Time per revolution	Space between drums	Type of drier	Air temperature (dry bulb)	Drying time	Protein	Fat	Moisture	
		lb.	sec.	in.		°F.	min.	pct.	pct.	pct.	
Group 1 Low fat, high protein, 0.25-inch holes in grinder plate	550B	100	65	.0625	Air-flotation	170-184	150	68.92	21.41	6.60	55.7
	521	100	65	.125	Air-flotation	134-160	180	65.47	20.18	12.45	40.0
	675	70	45	.0625	Air-flotation	160-180	120	66.91	19.63	9.90	35.2
	495	100	65	.125	Cabinet	140	240	63.03	20.42	14.13	34.5
	Mean	......	......	......	....	......	......	66.08	20.41	10.77	41.3
Group 2 Low fat, high protein, 0.875-inch holes in grinder plate	455	70	45	.091	Cabinet	180	240	69.92	18.88	8.98	34.5
	438	70	45	.091	Cabinet	180	180	64.95	19.63	13.08	31.5
	454	70	45	.25	Cabinet	180	240	70.05	18.54	9.06	30.8
	437	70	30	.091	Cabinet	180	180	64.71	19.37	13.78	26.7
	Mean	......	......	......	....	......	......	67.41	19.11	11.23	30.9
Group 3 High fat, low protein, 0.25-inch holes in grinder plate	674	70	45	.10	Cabinet	180	180	58.24	28.10	11.06	30.2
	584	70	45	.10	Cabinet	140	420	55.40	30.19	11.54	22.7
	582	70	45	.125	Cabinet	200	240	58.96	29.82	9.60	21.8
	586	70	45	.10	Cabinet	160	330	57.45	30.01	10.06	21.7
	Mean	......	......	......	....	......	......	57.51	29.53	10.57	24.1

through a 0.25-inch plate and the other through a 1.125-inch plate, were passed through a series of sieves, rehydration values obtained on particles of the two subsamples retained in any one sieve of a particular size mesh were relatively close. However, there was considerable variation in values obtained for particles of different sizes from any one sample. For example, the rehydration value obtained on the original ("composite") sample from a certain lot of dehydrated pork, for which the 0.25-inch plate had been used and which contained 54.7 per cent protein and 30.7 per cent fat, was 19.8 grams per 100 grams of product. When the sample was subdivided according to particle size, the values obtained for particles retained in sieves of 0.375-, 0.250-, and 0.125-inch mesh were 16.9, 20.9, and 26.5 grams, respectively. For a comparable lot, with which a 1.125-inch plate had been used, the rehydration value of the original ("composite") sample was 15.6 grams, whereas values obtained on particles retained in the same three sieves were 13.5, 17.3, and 21.0 grams, respectively. Similar results obtained on sieving five other samples of dehydrated pork also illustrated the fact that, for a given weight, smaller particles are more easily rehydrated than larger ones. The difference in behavior is believed to be due to the greater surface area of the former.

Another set of samples of dehydrated pork, 12 in number, all precooked on the double drum and then air-dried,[4] was subdivided according to relative proportions of protein and fat and to particle size as indicated by the grinder plate used. Rehydration was determined for all four samples in each of the three subgroups; the data are shown (Table 1).

It will be noted (Table 1) that Groups 1 and 2 contained about the same average proportions of protein, fat, and moisture but that for the former group a 0.25-inch grinder plate was used whereas for the latter the holes in the plate were 0.875 inch in diameter. When given the opportunity to rehydrate, samples in Group 1 absorbed considerably more moisture than those in Group 2. With particle size the same and moisture content practically so in Groups 1 and 3, comparison of these two throws further light on the effect of differences in protein and fat content or in the protein-fat ratio, the latter being 3.23:1 and 1.95:1, respectively. On the average, Group 1 samples took up about 70 per cent more moisture than Group 3 samples. It is readily apparent that the differences in protein and fat content occurring between Groups 1 and 3 of the same average particle size had a greater effect on rehydration capacity than the difference in particle size between Groups 1 and 2 where composition was practically the same.

In order to throw light on the effect, on rehydration capacity, of the searing or "case hardening" of the meat that appeared to occur during precooking on the double-drum machine, five lots of pork so precooked were compared with six others cooked in an open, steam-jacketed kettle. All lots were air-dried in a cabinet dehydrator; essential data are shown (Table 2).

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[4] Double-drum, cabinet, and air-flotation driers were described in the first article in this series, which appeared in *Food Research* 10, No. 5, 379-391.

TABLE 2

Influence of Type of Precooking on Rehydration Capacity of Cabinet-Dried Pork

Type of precooking	Sample No.	Precooking conditions		Drying conditions			Composition of dehydrated meat			Protein-fat ratio	Rehydration value (grams water per 100 grams meat)
		Time	Temperature	Type of drier	Temperature (dry bulb)	Drying time	Protein	Fat	Moisture		
		min.	°F.		°F.	min.	pct.	pct.	pct.		
Kettle	411	30	165	Cabinet	160	360	61.42	29.38	6.67	2.09:1	41.7
	412	30	212	Cabinet	160	305	60.49	29.78	7.40	2.03:1	38.2
	646	45	212	Cabinet	180	180	57.37	35.09	4.54	1.63:1	35.3
	652	45	212	Cabinet	180	150	56.74	34.69	4.75	1.64:1	34.2
	661	45	212	Cabinet	180	300	61.39	28.04	7.65	2.19:1	32.8
	769	30	212	Cabinet	180-160	210	64.10	26.12	7.24	2.45:1	43.8
	Mean	.....	......	....	......	.....	60.25	30.52	6.38	2.01:1	37.7
Drum	586	.....¹	.....	Cabinet	160	330	57.45	30.01	10.06	1.91:1	21.7
	617	.....	.....	Cabinet	180	300	56.96	31.47	9.46	1.81:1	24.1
	671	.....	.....	Cabinet	180	300	55.71	30.96	10.63	1.80:1	24.0
	673	.....	.....	Cabinet	180	300	57.08	30.59	9.89	1.87:1	24.4
	676	.....	.....	Cabinet	180	300	57.50	31.42	8.57	1.83:1	24.2
	Mean	.....	.....	....	......	.....	56.94	30.89	9.72	1.84:1	23.7

¹ Conditions for all drum samples: steam pressure, 70 pounds; time per drum revolution, 45 seconds; distance between drums, 0.10 inch.

TABLE 3

Influence of Processing Method on Rehydration Capacity of Dehydrated Pork

Processing method	Diameter of holes in grinder plate	Precooking conditions			Drying conditions			Composition of dehydrated meat			Rehydration value (grams water per 100 grams meat)
		Type of cooker	Temperature of product	Time	Type of drier	Air temperature (dry bulb)	Time	Protein	Fat	Moisture	
	in.		°F.	min.		°F.	min.	pct.	pct.	pct.	
Drum-air flotation........	.25	Double drum [1]	.....	.....	Air flotation	170	150	65.24	21.67	9.97	39.8
Kettle-air rotary............	.125 [2]	Steam kettle	212	30	Air rotary	[3]	.....	61.02	25.37	10.82	41.0
Kettle-cabinet..............	.25	Steam kettle	212	30	Cabinet	{ 180 160	{ 90 120	61.69	25.90	8.91	44.2
Vacuum-rotary..............	.25	Vacuum rotary	150	70	Vacuum rotary	108-140 (product)	280	64.03	27.04	5.73	56.3
Freezing-cabinet............	.25	Raw meat frozen then ground	.....	.....	Cabinet	120	300	64.58	27.98	3.76	60.0

[1] Seventy pounds of steam pressure, 45 seconds per revolution. 0.063 inch drum separation.   [2] Meat ground after cooking.   [3] Sixty-five pounds steam pressure, 25 seconds per revolution, in air-rotary dehydrator A.

Attention is directed to the fact that on the average the two types of product contained about the same percentage of fat but the protein content of the kettle-cooked meat was somewhat higher than that of the drum-cooked meat. Conversely, the former contained somewhat less moisture than the latter. This situation relating to protein and moisture content would appear conducive to somewhat smaller capacity on the part of the drum-cooked product to rehydrate. As shown (Table 2), it actually did absorb and retain less moisture than the kettle-cooked meat. However, the difference in rehydration capacity in favor of the kettle-cooked meat was so great one is led to the conclusion that the relatively small differences in composition were minor factors affecting water-absorbing capacity. Statistical analysis showed that the difference between the mean rehydration values of 23.7 and 37.7 grams was highly significant. There seems little doubt that the drum method of precooking meat for dehydration causes rather extreme denaturization of the protein and therefore does not compare favorably with the kettle method in its effect on the capacity of the finished product to take up moisture.

Comparison of processes was the major problem involved in the emergency meat-dehydration investigations. Rehydration capacity of the finished product was one of the factors through which the various processes were evaluated.

To throw more light on the value of different general methods of dehydration, a relatively large quantity of pork was divided into several lots of uniform composition. Each lot was then processed by a different method. Those processes selected for the comparison had shown good possibilities from one point of view or another in previous phases of the investigations. In each instance the process was carried out under conditions that earlier work had indicated to be practicable.

The methods studied and the conditions under which each was applied, as well as the protein, fat, and moisture contents of the dehydrated products and their respective rehydration values, are shown (Table 3).

The difference in rehydration values between any two products of the first three listed (Table 3) appears to be of little significance. On the other hand, the last two—vacuum-rotary and freezing-cabinet—although relatively low in moisture and for that reason probably possessed of a capacity to take up somewhat more moisture, were unquestionably highest in rehydration value. This was true despite the fact that they contained the greatest percentages of fat. The indications are strong that protein denaturization resulting from processing was less for the vacuum-rotary and freezing-cabinet methods than for any of the others.

SUMMARY

High-protein and low-fat content or a wide protein-fat ratio, small size of particle, and relatively low processing temperatures contributed to high rehydration values. Such values resulted from the use of the vacuum-rotary and freezing-cabinet methods. Relatively low rehydration values were obtained when the drum-air flotation, kettle-air rotary, and kettle-cabinet methods were employed.

CARBOHYDRATES IN CULTIVATED MUSHROOMS
(AGARICUS CAMPESTRIS)[1]

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Some investigational work has been carried out in other countries on the composition of mushrooms, but until recently very little information was available on the vitamin content or amount of other nutrients present in the cultivated mushroom *(Agaricus campestris)* grown in the United States.

Anderson and Fellers (1942) reported work on the vitamin, protein, and inorganic constituents of the cultivated mushroom. Cheldelin and Williams (1942) and Brunell, Esselen, and Griffiths (1943) also determined the amount of various vitamins present in this mushroom, while Fitzpatrick, Esselen, and Weir (1946) reported data on the composition of mushroom protein. This investigation was part of a project carried out in this laboratory to gather data on the nutritive value of the cultivated mushroom *(Agaricus campestris)* and was concerned with the carbohydrate fraction.

Anderson and Fellers (1942) reported the proximate analysis of this mushroom. Nickerson and Rettew (1944) isolated and identified mannitol from the same variety. They reported an average of 5.5 per cent of the dry weight of the immature button stage and 9.9 per cent of the dry weight of the mature open stage recovered as mannitol. Inagaki (1934) found mannitol present in wild mushrooms *(Agaricus campestris)* in concentrations of 0.93 per cent of the dry weight of the cap and 0.17 per cent of the stipe. So far as is known this is the only information available on the carbohydrate content of this variety of mushroom. Other carbohydrates and poly-alcohols, such as trehalose, glucose, glycogen, sorbitol, pentoses, and cellulose, have been reported present in other varieties.

EXPERIMENTAL PROCEDURE

The mushrooms used were grown in Massachusetts by a commercial grower. Good-quality, large, fresh mushrooms in the closed stage were selected, sliced into quarter-inch pieces, and dried in a forced-draft dehydrator at 65°C.(149°F.) for 12 hours. The dried product was ground in a Wiley mill to pass through a 60-mesh screen, then stored in hermetically sealed containers at 4°C.(39.2°F.) until used for analysis. The proximate analysis of the fresh, dehydrated, and moisture-free product is presented (Table 1).

Twenty-gram samples of the dehydrated product were extracted, first with anhydrous ethyl ether (16 hours reflux), then with 80 per cent ethyl alcohol according to the A.O.A.C. (1940) method for analysis of sugars in

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[1] Contribution No. 580, Massachusetts Agricultural Experiment Station.

plants. The alcohol-soluble extract and the residue were then tested separately for the presence of various carbohydrates by means of the common qualitative tests. By these tests many of the carbohydrates were found to be absent in the mushroom. Quantitative tests were then made for those carbohydrates whose presence might account for the positive qualitative tests.

TABLE 1

Proximate Analysis[1] of Mushrooms (Agaricus campestris)

Constituent	Fresh	Dehydrated	Moisture-free
	pct.	pct.	pct.
Water..	88.90	7.39	0.00
Protein (N × 6.25)............................	3.95	32.99	35.59
Fat (ether extract)............................	0.26	2.15	2.32
Extract matter (carbohydrate or starchy).....................	4.75	39.66	42.88
Fiber..	1.00	8.31	8.99
Ash..	1.14	9.50	10.22

[1] Proximate analysis made through the courtesy of P. H. Smith, Feed Control Laboratory, Mass. Agr. Expt. Station, Amherst, Mass.

All determinations were made on at least three samples and the analyses were run in triplicate. The results were calculated back to the fresh and moisture-free basis as well as for the dehydrated product; these results are presented (Table 2).

TABLE 2

Percentage of Various Carbohydrates and Poly-Alcohols in Mushrooms (Agaricus campestris)

Substance	Fresh (88.90 per cent water)	Dehydrated (7.39 per cent water)	Moisture-free basis
	pct.	pct.	pct.
Mannitol..	0.95	7.94	8.56
Reducing sugars (as dextrose)...................	0.28	2.30	2.48
Pentoses, methyl pentoses, hexuronic acids..........	0.04	0.30	0.32
Glycogen..	0.59	4.95	5.34
Crude hemicellulose...............................	0.91	7.59	8.18
Total..	2.77	23.08	24.88

Mannitol: The alcohol was driven off from the alcoholic mushroom extract by heating on a steam bath. Water was added from time to time until a concentrated solution, free from alcohol, was obtained. A water-insoluble precipitate was removed by filtration and then acetone was added to the filtrate in order to precipitate the sugars. The resultant precipitate was recrystallized from acetone several times and finally from absolute alcohol. The substance crystallized in pure white othorhombic needles, arranged in clusters. Identification was made according to Huntress and Mulliken (1941). The melting point was 164.8 to 166°C. (328.6 to 330.8°F.). No change was observed in a mixed melting-point determination using a known sample of recrystallized mannitol (Eastman). The substance had a sweet taste and did not reduce Fehling solution. The crude hexaacetyl compound had a melting point of 120.5 to 122°C. (248.9 to 251.6°F.); after

two recrystallizations from ether the melting point was 124.5 to 126°C. (256.1 to 258.8°F.). The hexabenzoyl derivative, precipitated from alcohol, had a melting point of 147.5 to 148.5°C.(297.5 to 299.3°F.).

Reducing Sugars: The reducing power of the alcohol extract was determined by the Lane-Eynon titration method, A.O.A.C. (1940), after the alcohol had been driven off and the solution cleared by the addition of neutral lead acetate. No increase in the amount of reducing substances was found after the solution had been heated in the presence of hydrochloric acid.

Glycogen: Glycogen was isolated from the alcohol-insoluble material by the A.O.A.C. (1940) method for meat products. The quantitative determination was made by hydrolyzing the product with hydrochloric acid and determining the resultant dextrose. The isolated material was a white amorphous powder which gave an opalescent solution. It did not reduce Fehling solution, gave no osazone precipitate, and produced a red coloration on the addition of an iodine solution.

Crude Hemicellulose: Crude hemicellulose was determined by the method of Weihe and Phillips (1942). The ground mushrooms were extracted first with a methyl alcohol-benzene mixture then with several portions of hot water. The material was then extracted with ammonium oxalate solution and analyzed for pectin, but no measurable quantity was obtained. The residue was partially delignified and the crude hemicellulose determined. The crude hemicellulose was analyzed and corrected for the presence of lignin, nitrogen, and ash.

Furfural-Yielding Substances: The method of Tollens and Kröber, as given by Browne and Zerban (1941), was used to determine the quantity of alcohol-soluble substances which yield furfural on heating with hydrochloric acid. These substances include pentoses, methyl pentoses, and hexuronic acids. The determinations were made on the cleared extract.

The reported mannitol content of *Agaricus campestris* is, in general, in agreement with the amount found in this same variety by Nickerson and Rettew (1944) but is much higher than that reported by Inagaki (1934) for wild mushrooms. There are no known data with which the other values can be compared.

SUMMARY

The principal carbohydrates and carbohydrate-like substances present in the cultivated mushroom *(Agaricus campestris)* were determined. Those present in the greatest quantities were mannitol, hemicellulose, glycogen, and reducing sugars. All together these accounted for 2.73 per cent of the weight of the fresh mushrooms.

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A STUDY OF BUTYRIC ACID-PRODUCING ANAEROBES
ISOLATED FROM SPOILED CANNED TOMATOES

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Spoilage of canned acid foods by butyric acid-producing anaerobes has attracted considerable attention in the past few years. Though canned acid foods are not usually as subject to bacterial spoilage as are nonacid foods, butyric acid-producing, sporeforming anaerobes are of particular economic interest in canning acid foods because of the short time these foods are processed and the comparative high resistance of the spores of these organisms in acid medium. This type of spoilage has been reported in tomatoes, pineapple, pears, apricots, and figs. The organisms studied in this paper were isolated from swelled canned tomatoes, and a comparison with previously described butyric acid-producing anaerobes from spoiled canned acid food was made.

Spiegelberg (1936) reported a species of *Clostridium* which caused bursting of canned pineapple. Cans in experimental packs inoculated with it did not swell when the pH was 4.4 or below. A group of butyric acid-producing, sporeforming anaerobes causing spoilage in acid foods was described by Townsend (1939). Five strains of these organisms isolated from tomatoes and from pears and figs in syrup were described. The organisms had high tolerance for acid and sugar and were believed to be quite similar to *Clostridium pasteurianum* (Winogradsky). Two types of organisms were studied, morphologically and physiologically, by Spiegelberg (1940a,b). He concluded they were quite similar and regarded them as smooth and rough strains of *Clostridium pasteurianum* (Winogradsky). Pure-culture inoculations with both types into commercially canned pineapple gave swells similar to those encountered in commercial packs.

EXPERIMENTAL PROCEDURE

Cultures used for this study were obtained from canned tomatoes. The cans were from different sources but all were hard swells, the contents of which, when opened, were frothy and full of gas. Original cultures from these cans were made in tryptone-dextrose-yeast extract broth stratified with tryptone agar after inoculation. This medium is recommended by the National Canners' Association for culturing organisms from spoilages of this type. It was found that these cultures would develop readily on the above medium which had been solidified by the addition of agar and incubated under anaerobic conditions in a Varny jar. Purification was accomplished by making streak plates from which representative colonies were picked. Thirty-one cultures were selected for study. Colonies on this medium were essentially of the S type, slightly convex, white to cream colored, round and smooth with an entire margin. Only a few so-called R type colonies were observed. They differed from the previously described

colonies only in that they had a rough surface and irregular outline. No great difference was observed in the growth of these later cells in broth, other than a tendency to settle out more rapidly. As a test for purity, streak plates were made on media and incubated aerobically. No growth resulted.

Before physiological tests were run on the cultures which had been isolated, various culture media suitable for anaerobic growth were tested. Stratification of media was considered bothersome, so various thioglycollate media were tried. Two fluid thioglycollate media (Brewer and Linden) were tested. Growth as evidenced by gas formation was slow and in seven cultures gas failed to appear during a week's incubation. Since the cultures grew well in the National Canners' Association medium, this formula was used with the addition of a small amount of agar and sodium thioglycollate. The formula for the stock medium was as follows:

Bacto-tryptone	10	grams
Dextrose	5	grams
Bacto-yeast extract	1	gram
K₂HPO₄	1.25	grams
Agar	0.5	gram
Methylene blue	0.002	gram
Sodium thioglycollate	1.0	gram
Distilled water	1,000	ml.

The reaction was adjusted to pH 6.8 and the medium was sterilized at 15 pounds pressure for 30 minutes. If not used immediately after sterilization, the tubes were exhausted for five minutes in flowing steam before inoculation. For stock cultures a small amount of calcium carbonate was added to the original medium before sterilization.

In order to determine whether physiological tests could be made in this thioglycollate medium, comparison was made using medium containing thioglycollate and the same medium without thioglycollate but stratified with agar. Duplicate tubes of each of the experimental media using dextrose as the test sugar were inoculated with the 31 cultures. In all cases growth and gas were produced readily in both media and in essentially the same time.

To further test the value of this thioglycollate medium for primary isolations from spoiled tomato products, tests were run in the two media using decimal dilutions of this product. In this way the relative ability of the two media to permit development of spores when present in decreasing numbers was tested. One milliliter of each decimal dilution was used as an inoculum. Eleven cans were chosen for this test. Cultures from nine of the 11 test cans gave the same results after five days' incubation; however, in cultures from five of these nine cans gas production was delayed 24 to 48 hours in the thioglycollate medium. In the other two test cans the 1-10,000 dilutions were positive in the stratified agar tubes, whereas the thioglycollate medium gave positive gas production in the 1-1,000 dilution but not in the 1-10,000 dilution.

Both Townsend and Spiegelberg have reported *Clostridium pasteuranium* as a significant spoilage organism in acid foods. In order to compare organisms isolated from the cans of spoiled tomatoes used in this study

with previously described spoilage organisms, cultures of *Clostridium pasteurianum* were secured from the American Type Culture Collection. Since *Clostridium butyricum* is a closely related, butyric-acid anaerobe, a culture of this organism was run for comparison. Following is the history of the cultures used:

No. 7040 *Clostridium pasteurianum* Strain 57
        Isolated from spoiled pineapple by Spiegelberg.

No. 7041 *Clostridium pasteurianum*
        Isolated from spoiled pineapple by Spiegelberg.

No. 6014 *Clostridium butyricum*
        Elizabeth McCoy, who received it from the Kral
        Collection in Vienna. Strain 97.

Cultures were received in liver-infusion, semisolid media with vaseline seal. Colonies obtained from plating these cultures were of the same types as colonies produced by spoilage organisms isolated in this work. Organisms from these colonies grew and produced gas in the thioglycollate medium.

MORPHOLOGICAL CHARACTERISTICS

Morphologically no difference between the 31 selections from the spoiled tomatoes and the cultures of *Clostridium pasteurianum* and *Clostridium butyricum* obtained from the American Type Culture Collection could be observed. All were rods with rounded ends, giving a positive gram reaction in young cultures which tended to become gram negative in older culture. The granulose reaction was positive, showing the characteristic blue violet stain. Spores were formed in the media. Dorner's spore stain indicater spores to be oval to round, usually terminal to subterminal. The cells were swollen during sporulation and in the cultures of *Clostridium pasteurianum* the spore envelope was retained longer than by the other cultures.

CULTURAL AND PHYSIOLOGICAL TESTS

Growth of all cultures was best at 25 to 30°C.(77 to 86°F.), slow growth occurred at 37°C.(98.6°F.), whereas no growth resulted when tubes were incubated at 55°C.(131°F.).

Litmus Milk Cultures: Whole milk (not homogenized) to which litmus was added was used. The cream was allowed to rise to the top to form a seal. Cultures from spoiled tomatoes and the culture of *Clostridium butyricum* produced acid, gas, and reduction in 24 to 48 hours with definite curd formation in 72 hours. Cultures 7040 and 7041 (*Clostridium pasteurianum*) gave only slight acid, gas, and reduction and formed no curd. Results from this test indicate a difference between the cultures of *Clostridium pasteurianum* and those isolated from the spoiled tomatoes examined in this work.

Gelatin: None of the cultures tested gave liquefaction in plain gelatin.

Fermentation Tests: For fermentation studies a basal medium was prepared, as previously described, with the exception that dextrose was omitted. It was tubed in nine-ml. amounts. This basal medium and the test materials were sterilized separately. Then one ml. of the material to be tested was added aseptically to the basal medium. All sugars and other

test materials were added so that the final concentration was one per cent. After the basal medium and test substance had been combined they were incubated 48 hours at 25°C. before inoculation to determine sterility. The medium was then inoculated with 0.5 ml. of the various cultures and incubated at 25°C. until positive results were obtained, or for two weeks, before being considered as negative. Positive results were turbidity with gas production. The results of these tests are shown (Table 1).

TABLE 1

Fermentation Studies on Cultures Isolated From Spoiled Tomatoes in Comparison With With Clostridium pasteurianum and Clostridium butyricum

Material tested	31 Cultures isolated from tomatoes	*Clostridium pasteurianum*		*Clostridium butyricum*
		No. 7040	No. 7041	
Arabinose	+[1]	—	—	+
Xylose	+	—	—	+
Dextrose	+	+	+	+
Galactose	+	+	+	+
Sucrose	+	+	+	+
Lactose	+	—	—	+
Maltose	+	+	+	+
Raffinose	+	+	+	+
Dextrin	+	±	±	+
Starch (soluble)	+	—	—	+
Inulin	+	+	+	+
Mannitol	+	+	+	+
Glycerol	—	+	+	—
Salacin	+	—	—	+

[1] + means growth with gas production; — means no growth, no gas production; ± means slight growth with small amount of gas production.

The cultures isolated from spoiled tomatoes fermented arabinose, xylose, lactose, salacin, and starch, whereas cultures of *Clostridium pasteurianum* did not attack these substances. Glycerol was fermented by *Clostridium pasteurianum* but not by cultures isolated from spoiled tomatoes. The fermentation reactions of *Clostridium butyricum* were similar to those of organisms causing spoilage in tomatoes. Mannitol fermentation by these organisms was less vigorous than that obtained from cultures of *Clostridium pasteurianum*. Other fermentation reactions were similar for all organisms tested.

Results of investigation reported in the literature has indicated that *Clostridium pasteurianum* can be differentiated from many of the other butyric-acid anaerobes on the basis of starch hydrolysis. To test this property a corn-mash medium was prepared consisting of five per cent yellow corn meal in water. This was sterilized and the tubes were sealed with sterile mineral oil after inoculation. Incubation was at room temperature and the cultures were held for two weeks before final readings were made. Cultures from spoiled tomatoes and *Clostridium butyricum* produced gas in 24 to 48 hours and had digested a large per cent of the corn starch at the end of two weeks. The two cultures of *Clostridium pasteurianum* produced no gas and no digestion of the starch during this period. These cultures were held two weeks longer with no change.

No attempts were made to determine the ability of these cultures to produce solvents or their ability to fix atmospheric nitrogen.

Tests for Spoilage of Tomato Products: To check the ability of these organisms to cause spoilage in tomatoes all cultures, including the two of *Clostridium pasteurianum* and the culture of *Clostridium butyricum*, were inoculated into tomato juice which was made anaerobic by means of a vaseline seal. Considerable variation in the time was required for the spoilage to occur. After the first five days one culture of *Clostridium pasteurianum*, the culture of *Clostridium butyricum*, and 20 of the 31 test cultures previously isolated produced growth as indicated by lifting of the vaseline seal by the gas produced. Six more cultures became positive after two weeks of incubation. The other five cultures and one culture of *Clostridium pasteurianum* were not positive at the end of a month. Subcultures made from these negative tubes into thioglycollate-yeast extract medium used in the previous tests gave good growth with gas production, indicating that spores were still present even though they had not developed.

Thermal Death Time Study: Of interest to packers of tomato products is the thermal death time of organisms causing spoilage in this product. To determine the thermal resistance of the cultures isolated from cans of spoiled tomato products eight cultures were chosen. They were grown in tryptone-dextrose-yeast extract agar containing calcium carbonate, under an anaerobic seal until a large number of spores were in evidence by microscopic examination. These cultures were then aged for one month in the culture medium before thermal death time tests were made. At this time spore suspensions were prepared in sterile water, heated to 80°C. (176°F.) for 10 minutes, and standardized so that they contained approximately 100,000 spores per ml.

Determinations were run in tomato juice having a pH of 4.4. In running heat resistances, the tomato juice in nine-ml. amounts was placed in a large Pyrex test tube and closed with a cotton stopper. This was placed in a water bath at the various temperatures used. After the juice had reached the proper temperature, one ml. of the spore suspension was added and uniformly mixed. At five-minute intervals one ml. of the tomato juice was removed and added to the basal thioglycollate medium. Tubes were incubated at room temperature; the final reading of results was made after two weeks' incubation. Three temperatures were used: 80, 90, and 100°C.(176, 194, and 212°F.); results are given (Table 2).

TABLE 2

Thermal Death Time of Organisms Isolated From Spoiled Tomatoes

Number of cultures	Temperature heated	Time in minutes						
		0	5	10	15	20	25	30
	°C.							
8	80	+[1]	+	+	+	+	+	+
8	90	+	+	+	+	+	+	+
5	100	+	+	—	—	—	—	—
3	100	+	+	+	—	—	—	—

[1]+ means growth in subcultures; — means no growth.

Results show that the organisms can withstand 80 and 90°C. for longer than 30 minutes since positive cultures were obtained from all the transfers made at these temperatures. At boiling temperature in tomato juice five of the cultures survived for five minutes but were destroyed in 10 minutes. Three of the cultures were slightly more resistant. The thermal death times obtained on these cultures are quite similar to those reported by Townsend (1939) and Spiegelberg (1940a) for cultures of *Clostridium pasteurianum* isolated from spoiled foods.

DISCUSSION

The literature reveals variable results on the cultural reactions of the butyric-acid anaerobes. In our tests with *Clostridium pasteurianum* received from the American Type Culture Collection and originally from Spiegelberg (1940a) the results obtained were identical with those reported by him for these cultures.

No attempt will be made to review the complete cultural reactions of this group of organisms but some characteristics will be discussed. Using lactose as the test sugar Waksman (1927) obtained fermentation with *Clostridium pasteurianum*, whereas Bergey, Breed, Murray, and Hitchens (1939), Spiegelberg (1940a), Townsend (1939), and Weinberg, Nativelle, and Prévot (1937) report negative results. McCoy, Peterson, and Hastings (1930) report both positive and negative results depending on the strain used. In our tests Cultures 7040 and 7041 (*Clostridium pasteurianum*) gave no fermentation. The 31 cultures isolated from tomatoes and Culture 6014 (*Clostridium butyricum*) fermented lactose.

Glycerol fermentation as reported by various workers is even more variable. Cultures 7040 and 7041 (*Clostridium pasteurianum*) fermented glycerol, which is in accordance with the results of Waksman (1927) and Spiegelberg (1940a). However, McCoy *et al.* (1930), Bergey (1939), Townsend (1939), Weinberg *et al.* (1937), and Bodily (1938) contend this organism does not ferment glycerol. Culture 6014 (*Clostridium butyricum*) and the cultures used in this work did not ferment glycerol.

Weinberg, Nativelle, and Prévot (1937) report that they obtained different reactions with some test substances when they used a peptone medium compared with an $(NH_4)_2SO_4$ medium. This may be a partial explanation of the variable results obtained by different workers. Another explanation of the variabilities may be that with some substances the production of gas is extremely slow. This observation was made during the cultural test run on these organisms; for example, in raffinose some of the cultures did not give positive results until after 120 hours of incubation.

Bergey (1939) indicates there is very little difference between *Clostridium pasteurianum* and *Clostridium butyricum*. The differentiating factor is that *Clostridium pasteurianum* does not ferment starch, whereas *Clostridium butyricum* does. Cultures 7040 and 7041 (*Clostridium pasteurianum*) gave negative results in soluble starch. The cultures isolated from spoiled tomatoes and Culture 6014 (*Clostridium butyricum*) gave positive results.

McCoy *et al.* (1930) believes that growth in corn mash is one of the most important cultural characteristics of butyric organisms. Their reports

indicate that *Clostridium pasteurianum* does not liquefy corn mash and produces only a small amount of gas. Our results on Cultures 7040 and 7041 (*Clostridium pasteurianum*) concur with the results reported by McCoy. The cultures isolated for this study and 6014 (*Clostridium butyricum*) produced gas rapidly and caused a digestion of the corn mash.

Throughout this ·comparative work only the one strain of *Clostridium butyricum* was used. The 31 cultures of butyric-acid anaerobes isolated from cans of spoiled tomatoes and used for this study agree closely with the morphological and physiological characteristics of this culture of *Clostridium butyricum*. They differ from the two cultures of *Clostridium pasteurianum* in their ability to ferment arabinose, xylose, lactose, salacin, and soluble starch. They also produced gas, acid curd, and reduction in litmus milk and digestion of corn mash with gas production, further characteristics which differentiate them from these later organisms.

SUMMARY

Cultures of butyric acid-producing, sporeforming anaerobes isolated from swelled spoiled canned tomatoes have been studied. A thioglycollate medium suitable for isolation and studying of cultural characteristics has been described. A comparison of these organisms with cultures of *Clostridium pasteurianum* and *Clostridium butyricum* has indicated that on the basis of cultural reactions the spoilage organisms isolated were more closely related to *Clostridium butyricum* than *Clostridium pasteurianum*. Thermal death time studies indicated that the spores produced by these organisms had a rather high resistance to heat under acid conditions.

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PECTINIC ACIDS AS RELATED TO TEXTURE AND QUALITY OF DEHYDRATED FRUIT PRODUCTS [1,2]

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The acceptance of dehydrated fruits and vegetables depends upon the general appearance, flavor, and eating quality of the rehydrated, cooked food. An index of quality which depends upon some constituent of the dehydrated food products would be desirable. An index of this nature could be used by the processor in improving his methods and it could serve as a guide to the wholesale purchaser. Deterioration* or the aging of a product could also be followed. The pectinic acids, as one constituent present in fruits or fruit products, may offer such an index of quality. Pectinic acids have been extracted, therefore, from several dehydrated products in order to investigate their possible relationship to quality. The results are recorded herein.

METHOD OF EXTRACTING PECTINS

The pectins were extracted from dehydrated fruits and vegetables by the method which produced an extraction liquor of the highest viscosity. It has been found that polyphosphates have a sequestering action for calcium which is advantageous when used in the extraction of pectin, Baker and Woodmansee (1944) and Maclay and Nielsen (1945). Since polyphosphates are valuable in the extraction of pectins above pH 3, sodium hexametaphosphate was used at its optimum concentration in the present experiments following a brief survey of the best conditions of acidity and boiling time for the extraction of pectin from each of several dehydrated products. The following example will serve to illustrate the survey procedure as applied to dehydrated apricots.

Twenty-gram samples of dehydrated apricots (blanched, dehydrated apricots, 1945 crop, held in cold storage) were soaked in 180 ml. of water for one hour and then broken down to a fine particle size in a Waring blendor through five minutes of blending action. The finely divided sample was extracted by boiling for five minutes with various amounts of sodium hexametaphosphate (0 to 5 per cent domestic Calgon) with or without the addition of acid (0 to 5 ml. N/1 HCL). In the case of this apricot sample the optimum condition for extraction, as determined from a series of six trials, was the presence of 2.5 per cent polyphosphate based on the weight

of the dehydrated fruit without added acid; the pH was 4.12. Under these conditions, an extraction liquor was obtained which had a relative viscosity (Ostwald) of 6.42 at 26°C.(78.8°F.).

Following the establishment of optimum conditions of acidity and poly-phosphate concentration as determined by optimum viscosity, additional samples were extracted under similar concentrations of extraction ingredients, but the time of extraction was varied from five to 15 minutes. When this was done in the case of this apricot sample, it was found that boiling 10 minutes produced the highest viscosity in an extract.

Since the actual grade of the pectin present in the dehydrated fruit or vegetable product was of primary interest, it was necessary to extract and prepare several grams of pectin from each dehydrated product. This was done by extracting 100 gm. of the dehydrated fruit. In the case of the same apricot example discussed in the above paragraphs, 100 gm. of the dehydrated apricot was soaked for one hour with 900 ml. of water containing 2.5 gm. domestic Calgon at 26°C., and then it was blended in the Waring blendor the same as the earlier, smaller-sized preparations. The blended product was boiled for 10 minutes, then the extract was strained and pressed through coarse muslin. The strained liquor was clarified by passing through a filter pad consisting of coarse filter paper and 60 gm. of Johns Manville Standard Super-Cel placed on a 10-inch Büchner funnel. A like amount of filter-aid was mixed with the strained liquor. Suction was applied to aid the clarifying filtration. The pectin in the clarified liquor was precipitated in twice its volume of 90-per cent isopropyl alcohol. Following precipitation the pectin was recovered by filtering, using closely woven muslin; it was pressed as dry as possible and then washed, in turn, with 100 ml. of the alcohol and 100 ml. of ethyl ether. The pectin was dried at 60°C.(140°F.) for 20 hours. The yield, methoxyl content, viscosity, and appearance of the pectin were recorded. The grades of the pectins were calculated from the viscosities of 0.5-per cent solutions by the formula— grade = 200 (log y — 0.2), where y = the viscosity of a 0.5- per cent solution of pectin.

<div align="center">RESULTS</div>

The pectins extracted from the dehydrated white potatoes, sweet pota-toes, beets, raisins, currants, prunes, and pears were all found to have low viscosity values; all except prunes had less than a relative viscosity of 2 for 0.5-per cent solutions at 26°C. One sample of seeded prunes had a relative viscosity of 3.65, but the other two samples had a viscosity of less than 3. These samples were considered worthless for purposes of correla-tion until further dehydrated products were evaluated.

Pectins extracted from samples of freshly dehydrated and from three-year-old dehydrated carrots had relative viscosities in one-per cent solution at pH 6.3 of 5.63 and 1.66, respectively. This would indicate grades of 55 and 2 when compared with the grades of pectins of commerce where 100-grade pectin is pectin which will support 100 pounds of sugar per pound of pectin as a 65-per cent soluble-solids gel of standard strength at its optimum pH of gelation. Upon giving the freshly-dehydrated carrots a rating of 100 as far as quality and texture were concerned, the three-year-old sample would rate about four. These ratings represent a good

index of the texture and quality of the dehydrated carrot samples at the time of examination.

The pectins obtained from the apple, apricot, and peach products were of higher grade and more suitable for correlation with the quality and texture of the prepared, dehydrated products. The results of their examination are given in the table. Correlations between the quality of fruit, which represents the averages of judgment by five individuals, and the grade of the pectin as calculated from viscosity are illustrated by the curves in the figure. A rating of 100 for quality is considered as very good, while 0 is considered to represent a worthless product.

TABLE 1

*Quality of Dehydrated Fruit as Related to Grade of Pectin
Present in Fruit Tissue*

Kind of fruit	Shelf history	Amount pectin extracted	Viscosity 0.5% solution 25°C.	Calculated grade of pectin	CH₃O	Quality of fruit (100= very good)
		pct.			*pct.*	
Apple..................	1944–cold storage	1.74	9.3	154	6.8	90
Apple..................	1942–cold storage	1.65	6.8	126	6.8	65
Apple..................	(?) '42 or '43	1.16	5.5	108	4.6	50
Apple..................	Pacific theatre	1.93	3.9	72	6.5	20
Apricot..............	1945–Dehydrated Fruit Assoc.	2.31	24.6	248	6.7	100
Apricot..............	1945–drum-dried	3.75	10.9	190	5.9	80
Apricot..............	1944–blanched, cold storage	3.42	7.2	132	6.8	75
Apricot..............	1945–blanched, cold storage	2.80	4.7	94	6.4	65
Apricot..............	1944–cold storage	4.44	4.2	84	6.1	45
Apricot..............	1944–vacuum-dried	3.17	3.9	78	5.9	30
Apricot..............	Pacific theatre	4.37	3.5	68	4.7	15
Peach..................	1944–blanched, cold storage	3.71	4.8	96	4.7	90
Peach..................	1944–cold storage	4.06	3.5	68	4.3	60
Peach..................	1944–cold storage	4.36	2.3	32	4.6	30
Peach..................	(?) Pacific theatre	4.03	2.3	32	3.4	10

The results show that there are good correlations between pectic quality and product quality for these particular fruits—apples, apricots, and peaches. Some level of acceptability has to be taken for the quality of fruit. In practice this might be 60. Below this level of acceptability the dehydrated products are worthless from the edibility standpoint. It can be assumed that the viscosity of pectin extracted from fruits will be a good index of edibility. This is because the viscosity of pectin solutions of constant concentration, in this case 0.5 per cent, is an index of degree of polymerization of the pectin. The degree of polymerization of pectin appears to be a good index of the treatment and conditions of storage which the pectin has undergone whether it is still present in vegetative tissues or extracted from such tissues and held in liquid or powdered form. It is unnecessary to convert the viscosity values to grade except in order to° convey a better conception of quality of the pectin to the reader who is interested in production and who is unfamiliar with viscosity as a term.

Straight lines are found when the values for apples and peaches are plotted. The figure shows that the values for the apricots do not produce

a smooth curve; however, when grade is multiplied by methoxyl content of each particular pectin then a smoother curve is obtained. Pectin loses its methyl ester content at a rate depending upon storage conditions of moisture, temperature, and acidity. It is possible, therefore, to have two indices of deterioration—grade and methoxyl content. However, grading, or viscosity, appears to be a better index when the results presented at this time are considered.
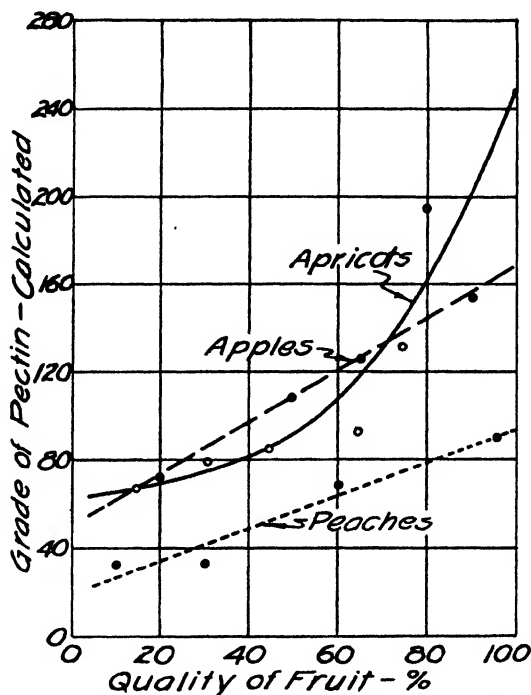
Fig. 1. Quality of dehydrated fruit as judged by grade of pectins present in its tissues.

CONCLUSION

A study of pectinic acids as related to the texture and quality of dehydrated fruits and vegetables has shown that grade (as calculated from viscosity) of pectins present in the dehydrated products may be an index to their quality. A straight-line relationship was found in the case of apples and peaches but not with apricots. Dehydrated carrots, apparently, can be graded in this same manner. The viscosities of solutions of pectins obtained from samples of dehydrated white potatoes, sweet potatoes, beets, raisins, currants, prunes, and pears were too low for correlation with eating quality.

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EVALUATION OF CAROTENE CONTENT OF FRESH AND COOKED SPINACH [1]

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It has been found in this laboratory, Porter, Wharton, and Beltz (1944), that certain vegetables, after cooking, have more carotene per 100 grams dry weight than the corresponding fresh vegetables. Booher, Hewston, and Marsh (1941), examining leafy vegetables and green beans; MacLeod and Utley (1937) and MacLeod (1939), sweet potatoes; and Richardson and Mayfield (1940), peas, found more carotene in the cooked product than in the fresh whether determined by biological or chemical methods. This has been explained as resulting from (1) destruction of carotene owing to enzymatic action in the fresh vegetables; (2) greater availability of carotene to the animal by the increased digestibility of the cooked product; and (3) high values for carotene owing to the carotenoid pigments included in the carotene extracted from cooked vegetables. It is difficult to believe that increases in carotene concentrations of 30 per cent in cooked vegetables can be explained either as increased availability or as errors of analysis.

Most investigators have calculated the nutrients of fresh and processed vegetables per unit of dry weight of vegetable. This method of evaluation may result in misleading interpretations which are more evident with the slightly soluble nutrients, not appreciably destroyed by cooking. In this procedure it is usual to use drained weight of cooked vegetable and discard the cooking liquid. A transfer of nutrients to the cooking liquid in processing may occur either by solution or by washing from the broken plant cells. Usually the proportion of water to vegetable is relatively large, so that, although a considerable amount of the nutrient may be in the cooking water, it becomes impractical to determine the concentration. It frequently is difficult to drain many cooked vegetables quantitatively so the cooked weight upon which the calculation of per cent of solids is based may be unreliable. Moreover, the methods for determination of moisture are empirical. Plant material is colloidal in nature and, therefore, it is likely that its moisture determination by routine laboratory methods does not include all of the water, since a fair percentage of the moisture is bound water, not readily removed. Cooking breaks down this colloid structure and water may no longer be retained as bound water on drying.

Benne (1942) stated that carotene values, if expressed on the water-free basis, should permit a comparison of relative concentrations within different samples of fresh crop plants if the moisture contents could be determined with perfect accuracy. If an actual moisture content of 90 per cent is assumed, however, an error of one per cent in its evaluation would cause a variation of 10 per cent in the carotene value expressed on a relative-weight basis, and in this way differences in carotene content really caused by other factors might be concealed.

Since the apparent increased concentration of carotene in cooked vegetables has been inadequately explored, the problem reported here was undertaken.

EXPERIMENTAL PROCEDURE

Spinach used for this experiment was purchased on the local market during the months of December, January, February, and June. Leaves of approximately the same size were washed in distilled water, dried with cheesecloth, and the petioles and midribs removed. Leaves from each lot were clipped with shears into approximately one-centimeter squares, dropped into a paper bag, and shaken to mix. Samples were weighed at once and analyses made in duplicate.

A four-serving portion of greens (400 gm.) was cooked in twice its weight of boiling water in a three-quart enamelled saucepan. Five- and 10-gram samples for carotene and moisture tests, respectively, were put in bags of cheesecloth, parchment, and perforated parchment and cooked in the saucepan with the four-serving portion (Table 1). A second experiment was conducted in which fresh samples were cooked for 15 minutes in twice their weight of water in sealed, impervious, pliofilm bags. Moisture and carotene determinations were made on both the entire contents of the bag and the drained solids (Table 2). Samples from the cooked four-serving portion were taken by means of glass boring tubes, one-half inch in diameter, pressed through a well mixed and compact layer of greens, and their weights were determined. The small samples which were cooked with the four-serving portion were drained as nearly as possible to the same degree as the large one and the cooked weights obtained. Moisture was determined on all samples by drying at 95 to 105°C.(203 to 221°F.) for 24 hours.

The Petering, Wolman, and Hibbard (1940) procedure, modified to use a Waring blendor for producing a fine dispersion of the plant material in absolute alcohol, was used to measure carotene. The standard carotene curve was made with a mixture of 90 per cent beta and 10 per cent alpha (S.M.A. Corp.) carotene.

Correlation coefficients were determined for the fresh and cooked carotene values and, since these values are correlated, a linear regression was obtained. For the regression equation $y = cs$, the regression coefficient c was obtained from the formula $C = \dfrac{\Sigma xy}{\Sigma x^2}$ where $y =$ mg. of carotene per 100 grams of dry weight of fresh spinach and $x =$ mg. of carotene per 100 grams dry weight of cooked from the same lot. The "t" values for correlated data also were calculated.

RESULTS AND DISCUSSION

The effects of the various cooking processes upon the carotene content of spinach are tabulated (Table 1). Treatments 1 to 5, inclusive, were done with spinach purchased in January and February; Treatments 6 and 7 were December samples. Each treatment was replicated six times, duplicate tests being made on each replication. The first and second columns present the carotene concentration of the raw and cooked samples, respectively, calculated per 100 grams of dry weight using the value for total solids determined for each case; the third column shows the per cent apparent change in carotene of the cooked sample; the fourth, the carotene values of the same samples calculated on their uncooked weight; and the fifth, the per cent difference of this value from that of the original fresh sample. Columns 6 and 7 give the total solids of the fresh and cooked samples. The mean errors of the average values for carotene and solids also are included.

The carotene concentration of the cooked four-serving portion (Treatments 1 and 6) showed highly significant gains over that of the corresponding fresh vegetable when the dry weight of the fresh and cooked vegetable, respectively, were used for calculation.

Since the cooked weight could be affected by the amount of water left in the product after one minute of draining in the colander, the cooked spinach was drained with suction on a Büchner funnel. Complete drainage could not be obtained because this method still did not remove the water trapped between the leaves, even though the spinach was cut and turned while suction was being applied. The results obtained (Treatment 2), although less consistent, showed significantly higher carotene concentrations in the cooked vegetable.

Halliday and Noble (1943) suggested that small samples of vegetables be weighed into cheesecloth bags and cooked with a large sample. This was done in Treatment 3; after cooking, the samples were drained and weighed.

Inasmuch as the sample could not be removed quantitatively from the bag, the cheesecloth had to be included in this weight and the weight corrected. Carotene was determined after the sample and bag were extracted in a Waring blendor and the results were not significantly different from those obtained on fresh spinach. That the results were not too consistent is shown by a mean error of 6.1 and 5.1, respectively.

For Treatment 4, the weighed samples of fresh spinach were put into finely perforated parchment bags and cooked with the four-serving portion. Since the perforations of the parchment permitted free interchange of liquids inside and outside the bag, the cooking conditions should be identical with those of the large sample. The carotene was calculated on the basis of the solids in both the fresh and cooked sample as in Treatment 3. The average carotene content calculated from the cooked weight of vegetable showed a gain of 33 per cent above that of the fresh value, which was a significant increase. When carotene was calculated from the weight of fresh vegetable placed in the bag, there was an average loss of 2.5 per cent. These results indicate that the cooking process in itself does not

TABLE 1

Carotene Content and Total Solids of Fresh and Cooked Spinach[1]

Treatment	Carotene per 100 grams dry weight					Total solids	
	Fresh	Cooked	Difference	Calculated on uncooked weight	Difference	Fresh	Cooked
	mg.	mg.	pct.	mg.	pct.	pct.	pct.
1. 400 gm. cooked and drained one min. in colander	57.4±3.5	78.0±3.3[3]	+35.8	....	....	8.7±0.5	7.0±0.3
2. 400 gm. cooked and drained in Büchner funnel	57.4±3.5	75.8±4.7[3]	+32.0	....	....	8.7±0.5	7.8±0.4
3. Five gm. cooked in cheesecloth bag[2]	57.4±3.5	47.3±6.1	−17.6	47.1±5.1	−18.0	8.7±0.5	8.1±0.9
4. Five gm. cooked in perforated parchment bag[2]	57.4±3.5	76.4±6.9[4]	+33.1	56.0±3.5	− 2.5	8.7±0.5	8.7±0.5
5. Five gm. cooked in parchment bag; drained, liquid discarded; carotene determined on solid portion[2]	57.4±3.5	71.0±6.4	+23.7	56.7±3.1	− 1.2	8.7±0.5	8.4±0.4
6. 400 gm. cooked and drained one min. in colander	41.4±1.8	54.6±2.3[3]	+31.9	....	....	11.4±0.4	8.8±0.2
7. Five gm. cooked in parchment bag; entire contents analyzed[2]	41.4±1.8	....	....	39.4±2.1	− 4.8	11.4±0.4	....

[1] Each figure represents the average of six replications in duplicate. [2] Small samples were cooked with the four-serving portion. [3] The probability that this is a chance variation from the fresh value is 1/100. [4] The probability that this is a chance variation from the fresh value is 1/20.

increase the carotene content, but the increase is an apparent one owing to the fact that the weight of the cooked and fresh product are not comparable either before or after drying.

In Treatment 5, the spinach was cooked in parchment bags, drained, the liquid discarded, and the carotene determined in the drained solids. The results were similar to those of Treatment 4. Difficulty in obtaining uniform drainage is indicated by the fact that the carotene values calculated on the fresh weight have a mean error of 3.1 and on the cooked weight, 6.4.

TABLE 2

Carotene and Total Solids of Spinach (Five-Gram Sample)
Cooked in Sealed Pliofilm Bags

Treatment	Carotene per 100 grams dry weight					Total solids	
	Fresh	Cooked	Differ-ence	Calcu-lated on uncooked weight	Differ-erence	Fresh	Cooked
	mg.	mg.	pct.	mg.	pct.	pct.	pct.
1. Entire contents analyzed.......	55.7[1]	53.5[1]	— 4.0	52.2[1]	—6.3	9.2[1]	8.9[1]
2. Drained contents analyzed....	55.7[1]	75.7[2,3]	+35.8	53.5[2]	—4.3	9.2[1]	7.2[2]

[1] Represents the average of four replications in duplicate.   [2] Represents the average of three replications in duplicate.   [3] The probability that this is a chance variation from the fresh is 1/100.

In Treatment 7, weighed samples of fresh spinach were placed in parchment bags and cooked with the four-serving portion. The entire contents of the bag were analyzed for carotene. These results were 4.8 per cent lower than those obtained with the fresh spinach, which was. not a significant difference. Because the various treatments reported (Table 1) indicated that the gain in carotene was apparent rather than real, further work is reported (Table 2). Since pliofilm is impervious to water, it is possible to cook small samples in sealed pliofilm bags in an amount of water proportional to that used for the 400-gram sample. The cooking conditions are not identical to those of the four-serving portion since the contents of the bag had contact only with trapped air which may or may not be important.

When the entire content of the bag was analyzed, the carotene of the cooked spinach was not significantly different from that of fresh, regardless of whether the total solids of the fresh or cooked portion were used in calculation. In the cooked samples, when the liquid was drained off and the spinach weighed and analyzed, there was a 36-per cent gain in carotene over that of the fresh, which was significant. When the latter determinations were calculated on the basis of the fresh weight of spinach, however, there was no gain in carotene. These data further confirm the observation that there is no actual increase in the carotene owing to cooking and that no significant amount is washed into the cooking water, but that the apparent gain is due to a difference in the solids of the fresh and cooked vegetable. It is apparent (Table 2) that approximately a fifth of the total solids of spinach are lost in the cooking water. Where the cooking water is included in the determination of solids, however, the value is still lower than that found in fresh spinach. In 12 cooking trials, where per

cent total solids of the cooking water and of the cooked vegetable were determined independently, the sum of the two in no case equalled the total solids determined for a 100-gram portion of raw vegetable. Differences varied from two to 19 per cent. Thus, even though the cooking water is analyzed and the solids lost in this way considered in the calculation, a procedure which is not always practical and not routinely practiced, dry weight is a variable measure dependent upon the physical structure of the product dried.

Benne (1942) pointed out that a small change in total solids would produce a much greater change in the apparent concentration of a nutrient such as carotene which occurs in small amounts. It would appear that this artifact would apply to all nutrients and not just to those which are water insoluble and not destroyed appreciably by ordinary cooking procedures. However, to demonstrate this, it will be necessary to consider both the concentration in the cooking water and the destruction during cooking.

A correlation coefficient of 0.833 was found between the average carotene content of all samples of fresh and cooked spinach when expressed in terms of dry weight of vegetables. The linear relationship ($y = ex$) of the carotene content of the fresh spinach on that of the cooked is shown (Fig. 1). The regression coefficient is 0.761 and the error of estimate 5.16 mg. If the obtained carotene concentrations of the cooked vegetable are multiplied by 0.761, the values are no longer significantly different from the fresh.

Since the data (Tables 1 and 2) have shown that any small variation in the determination of total solids causes a much greater difference in the carotene content, it was considered advisable to compare the raw and cooked carotene concentrations per 100 grams moist weight. The linear regression of the carotene in fresh on that of cooked spinach is shown (Fig. 2). The regression coefficient is 0.897, the error of estimate 0.706 mg., and the correlation coefficient 0.926. Fifty per cent more of the cooking trials fell within the area bounded by $\pm$ 1 error of estimate when moist weight was used as a basis for calculation. It would seem not only that it is unnecessary to do moisture determinations in order to make comparisons between raw and cooked samples but if the relationship between the carotene in the raw and cooked vegetable is once established, carotene concentration of the cooked product may be predicted more precisely from the carotene of the raw vegetable than it may be determined if expression of the result depends on the measure of dry weight.

The regression for each type of processing would need to be established. However, if this relationship were known, fresh material could be analyzed and information obtained concerning the cooked product by applying a constant obtained by the relationship $\dfrac{\Sigma xy}{\Sigma x^2}$. For example, in the case of the described method for home-cooked spinach, this factor would be 1.30.

Since there were data on green beans and Swiss chard available from earlier studies in this laboratory, similar analyses of the relation between the carotene of the raw and cooked vegetables were made; the results are recorded (Table 3). In all the Swiss chard data six grams of sodium chloride for each four-serving portion were used in the home-cooking pro-
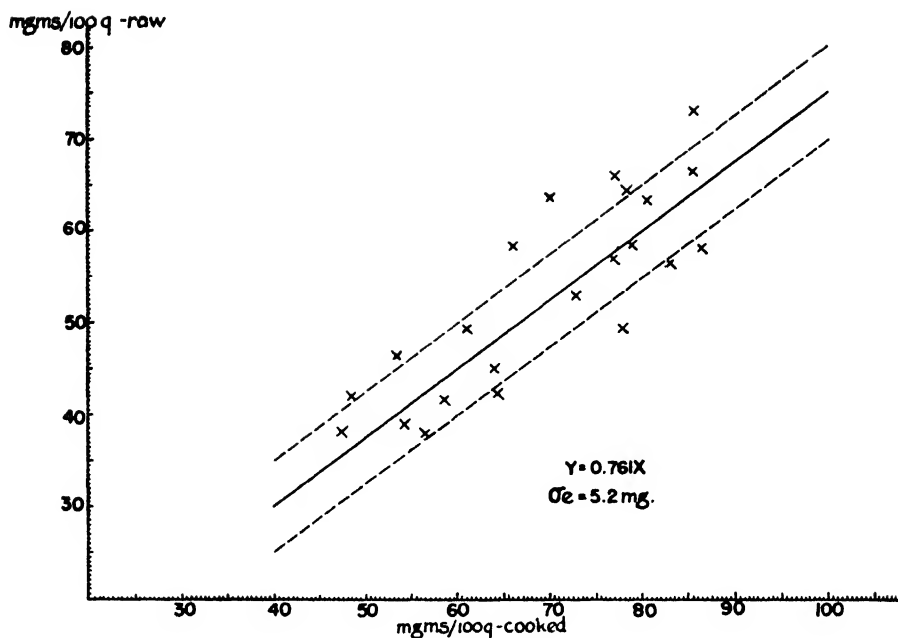
Fig. 1. Regression of carotene concentration in milligrams per 100 grams dry weight of raw and cooked spinach. (× represents actual values obtained.)
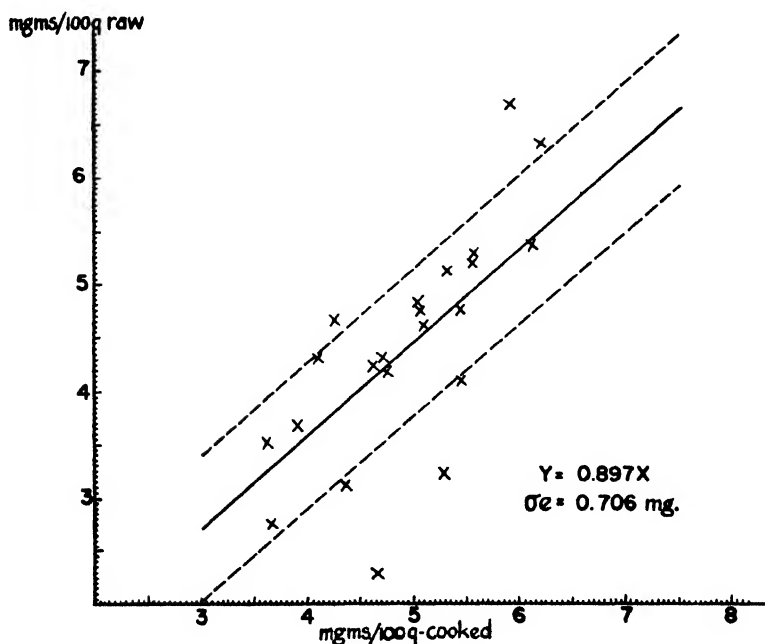
Fig. 2. Regression of carotene concentration in milligrams per 100 grams moist weight of raw and cooked spinach. (× represents actual values obtained.)

cedures, and for the institution cooking, 9.1 grams for each 30 servings. The canned green beans also contained salt but not the other processings. In most cases there were only four replications of triplicate determinations and this could scarcely be considered sufficient data for conclusions. However, in every case but one (the holding of institution-cooked Fordhook chard on the steam table for one hour) the value obtained by applying the constant was closer to that of the fresh than the cooked value calculated from the dry weight.

TABLE 3

Factors for Adjusting Carotene Values of Processed Vegetables

Vegetable	Number of replications [1]	Carotene before processing per 100 grams dry weight	Type of processing	Carotene after processing per 100 grams dry weight	Regression coefficient	
					Fresh value on cooked	Cooked value on fresh
Spinach (local market)	22	*mg.* 53.2	400 gm. cooked in twice its weight of boiling water	*mg.* 69.6	0.761	1.30
Green beans (Landrettes stringless green pod)	4	2.94	Steam blanched, 174°F. for 2.5 min.	3.23	0.905	1.10
	4	2.93	Steam blanched, 208°F. for 3.75 min.	3.26	0.907	1.09
	8	3.41	Canned—boiled 10 min.	3.68	0.928	1.07
	8	2.90	Frozen—boiled 12 min.	3.25	0.891	1.12
Fordhook chard	4	62.9	(1) 6 gm. NaCl added then cooked in twice its weight of boiling water	66.0	0.942	1.05
	4	62.9	(2) 6 gm. NaCl added then cooked in water clinging to leaves after last rinsing	61.2	1.03	0.973
	4	62.1	(3) 8 lb. cooked in steam-jacketed kettle (9 gm. NaCl added after cooking), held on steam table 10 min.	69.0	0.893	1.11
	4	62.1	(4) Same as (3), held on steam table for 1 hour	62.1	0.983	1.00
Rhubarb chard	4	57.1	Same as Fordhook (1)	70.8[2]	0.802	1.24
	4	57.1	Same as Fordhook (2)	60.6	0.938	1.06
	4	58.6	Same as Fordhook (3)	74.1[2]	0.784	1.26
	4	58.6	Same as Fordhook (4)	72.1[3]	0.811	1.23

[1] Each replication is the average of triplicate determinations except the spinach which is in duplicate. [2] The probability that this is a chance variation from the fresh value is 1/100. [3] The probability that this is a chance variation from the fresh value is 1/20.

Respective regression coefficients of the carotene of fresh vegetable on the cooked were quite different although both chard and spinach are leafy vegetables. The coefficient apparently varies with the type of vegetable, the variety, and the processing.

SUMMARY

Carotene in spinach increased over 30 per cent above the fresh values when cooked by a standard home procedure and calculated as milligrams per 100 grams dry weight of vegetable. Evidence is presented that this was not an actual increase in carotene but an apparent one owing to the difference in the solid contents of the fresh and cooked products. Including the solids from the cooking water in the total solids of the cooked spinach accounted for only a portion of the difference. A linear relationship was found between the carotene content of the fresh and cooked spinach. A regression coefficient can be calculated for the carotene of fresh and cooked spinach for either the moist or dry vegetable which would permit a more accurate evaluation of the carotene content of the cooked vegetable. After this constant is established, a carotene determination need only be done on either the fresh or the cooked product.

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GROWTH OF *STAPHYLOCOCCUS AUREUS* IN VARIOUS PASTRY FILLINGS

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Foods, such as dairy and meat products, offer suitable media for the growth of pathogenic bacteria and so may be responsible for spreading bacterial food poisoning of the infection and toxin types.

It is known that staphylococci are widespread in nature and may cause a toxin type of food poisoning after gaining entrance to practically any type of food which supports their growth. Dack (1943) refers to this toxin type of food poisoning as probably the most common of all.

This widespread occurrence of staphylococci, together with many food-poisoning outbreaks during the past 40 years that were attributed to staphylococci and that followed the consumption of milk products, cream fillings of the éclair type, custards, or cakes with cream fillings, has led a number of investigators to undertake work involving the growth and control of staphylococci in various food products. Some early work was reported by Stritar, Dack, and Jungewaelter (1936), who successfully killed staphylococci in custard-filled puffs and éclairs by reheating these products without impairing their flavor or appearance. Jones and Loch-·head (1939) showed that the growth of enterotoxin-producing staphylococci was inhibited at 4.4°C.(40°F.).

Cathcart, Ryberg, and Merz (1942) effectively reduced the counts of *Staphylococcus aureus* and *Salmonella enteritidis* in the air by utilizing ultraviolet rays (2,000 to 2,950 Å units). The same authors, Cathcart, Merz, and Ryberg (1942), also showed that *Staphylococcus aureus* and *Salmonella enteritidis* were eliminated from experimentally inoculated custards which had been brought to a second boil.

Ryberg and Cathcart (1942) found acidity in pure fruit fillings to be effective in inhibiting the growth of *Staphylococcus aureus* and *Salmonella enteritidis*. Segalove, Davison, and Dack (1943) also found the growth of *Staphylococcus aureus* to be best in low-acid foods (peas, corn) but retarded in high-acid foods (peaches, tomato juice). Chocolate and natural cocoa fillings, made according to the formulae outlined by Cathcart and Merz (1942), were shown to exert an inhibiting influence on the growth of *Staphylococcus aureus*.

This paper deals with the results obtained from investigating the growth of *Staphylococcus aureus* in commercial dry-mixed puddings (with and without the addition of milk); prepared vanilla fillings; pie fillings made from pumpkin, squash, and sweet potato; fruit fillings; cheese cake fillings; and whipping-cream-type mixes. These fillings and mixes were prepared to serve as "bacteriological media." Some were modified by the addition of various amounts of citric acid, lactic acid, and sodium propionate.

EXPERIMENTAL PROCEDURE

The formulae of the various fillings and mixes used were as follows:

1. *Standard Custard Filling.* The standard custard used for comparison counts was made as follows, according to the formula of Cathcart, Merz, and Ryberg (1942):

Water—1 qt.   Cornstarch—2½ oz.
Sugar—8 oz.   Eggs (whole)—2
Powdered whole milk—4 oz.   Salt—⅛ oz.

Three-fourths of the water, one-half of the sugar, and all of the powdered milk were mixed and brought to a boil. The remainder of the sugar was "dry mixed" with the cornstarch; the eggs and salt were added and made into a paste with part of the water. This paste was thinned with the remaining one-fourth of the water and added to the mixture which had been brought to a boil. The completed mixture was boiled for one minute.

2. *Vanilla Filling*

Water—1 lb., 8 oz.   Frozen yolks—2⅔ oz.
Sugar—10 oz.   Cornstarch—2 oz.
Salt—⅛ oz.   Water—8 oz.
                                   Vanilla flavor—⅛ oz.

The water, sugar, and salt were brought to a boil. The frozen yolks, cornstarch, water, and vanilla flavor were mixed and added to the boiling mixture with constant stirring until the entire mixture had been brought to a second boil and then boiled two minutes.

3. *Vanilla Filling*

Water—1 lb., 8 oz.   Water—8 oz.
Sugar—10 oz.   Vanilla flavor—1/16 oz.
Frozen yolks—4 oz.   Butter—3 oz.
Cornstarch—4 oz.   Emulsified shortening—3 oz.

The same procedure as used for No. 2 vanilla filling was followed for this filling except that the butter and shortening were added during the first boil.

4. *Pumpkin Pie Filling*

Sugar—6 oz.   Nutmeg—0.9 gm.
Pumpkin—1 lb.   Cardamon—0.45 gm.
Whole eggs—2 oz.   Water—1 lb., 3 oz.
Yolks—2½ oz.   Milk powder—3 oz.
Salt—⅛ oz.   Glucose—2½ oz.
Cornstarch ⅝ oz.   Butter—⅜ oz.
Allspice—0.9 gm.

The sugar, pumpkin, eggs, salt, cornstarch, and spices were mixed. To this mixture was added the glucose and milk powder dissolved in water and last the butter, melted. The mixture was thoroughly stirred and then baked, uncovered, at 232.2°C.(450°F.) for 30 minutes.

5. *Pumpkin Pie Filling*

Sugar—6 oz.   Allspice—0.9 gm.
Pumpkin—1 lb., 3 oz.   Nutmeg—0.9 gm.
Whole eggs—2 oz.   Cardamon—0.45 gm.
Yolks—2½ oz.   Water—1 lb., 3 oz.
Salt—⅛ oz.   Glucose—2½ oz.
Cornstarch—⅝ oz.

The procedure for mixing No. 5 pumpkin pie filling was the same as that for No. 4.

6. *Sweet Potato Pie Filling*

Sugar—8 oz.   Yolks—1½ oz.
Salt—⅛ oz.   Sweet potato pulp (canned)—
Water—1 lb., 3 oz.   1 lb., 3 oz.
Glucose 2 oz.   Melted butter—⅜ oz.
Whole eggs—1½ oz.

The method of preparation was the same as that for pumpkin pie fillings.

7. *Squash Pie Filling*

Sugar—5 oz.	Cloves—⅛ gm.
Canned squash—14 oz.	Nutmeg—¼ gm.
Whole eggs—3 oz.	Water—1 lb., 3 oz.
Yolks—1½ oz.	Glucose—2 oz.
Salt—1/16 oz.	Melted butter—¼ oz.
Cornstarch—5/16 oz.	

The method of preparation was the same as that for pumpkin pie fillings.

8. *Peach Filling*

Water—1 qt.	1 lb., 8 oz.
Sugar—1 lb., 8 oz.	Cornstarch—3 oz.
Salt—⅛ oz.	Egg yolks—2 oz.
Canned peaches (ground)—	

9. *Raspberry Filling*

Water—1 qt.	1 qt. (dry measure)
Sugar—1 lb., 8 oz.	Cornstarch—3 oz.
Salt—⅛ oz.	Egg yolks—2 oz.
Fresh raspberries (ground)—	

In preparing the peach and raspberry fruit fillings, three-fourths of the water and one-half of the sugar were mixed and brought to a boil. The fruit was added to this boiling mixture. The remainder of the sugar was "dry mixed" with the cornstarch, egg yolks, and salt and made into a paste with part of the water. The paste was thinned with the remainder of the one-fourth quart of water and added to the mixture which had been brought to a boil. The completed mixture was boiled for one minute.

10. *Cheese Cake Filling*

Baker's cheese—1 lb.	Water—2⅔ oz.
Cornstarch—1½ oz.	Whole eggs—5 oz.
Emulsified shortening—1⅜ oz.	Egg whites—3 oz.
Sugar—8 oz.	

The water, whole eggs, and egg whites were mixed and stirred for several minutes. The cheese, cornstarch, shortening, and sugar were then added and the mixture was stirred thoroughly to a mass of smooth consistency. It was then baked, uncovered, at 232.2°C. (450°F.) for 30 minutes.

11. *Whipping-Cream-Type Mix*

Sweet butter—1½ lb.	Skim-milk powder—14 oz.
High-ratio vegetable	Sugar—10 oz.
shortening—1½ lb.	Water—72 oz.

12. *Whipping-Cream-Type Mix.* Same as No. 11, except the butter was replaced with high-ratio vegetable shortening.

13. *Whipping-Cream-Type Mix*

Sweet Butter—1½ lb.	Fresh whole milk—2½ qt.
High-ratio vegetable	Skim-milk powder—3 oz.
shortening—1½ lb.	Sugar—10 oz.

14. *Chocolate Whipping-Cream-Type Mix.* Same as No. 11, except 12 ounces of chocolate liquor were added.

The following general procedure was used in combining the ingredients in all the whipping-cream-type mixes: The powdered milk was dispersed in the water or fresh whole milk, the fat added in small pieces, and the mix

heated to 62.8°C.(145°F.). When the temperature reached 145°F., the heating was discontinued and the mix was stirred until the melted fats were evenly incorporated. The mix was then strained, homogenized, pasteurized by holding at 65.6°C.(150°F.) for 30 minutes, and then cooled to 1.7 to 4.4°C.(35 to 40°F.).

All of the filling and mix preparations were made in duplicate. One of each pair (except the whipping-cream-type mixes) was autoclaved at 15 pounds pressure for 15 minutes. One preparation of each whipping-cream-type mix was heated in an autoclave to 15 pounds pressure, but as soon as this pressure was reached, the autoclave was shut off. All fillings and mixes were refrigerated prior to their inoculation with the organism *Staphylococcus aureus*. In most cases, a comparison was made of the growth of *Staphylococcus aureus* in both the autoclaved and unautoclaved fillings. Since growth of the organism was comparable in both the autoclaved and unautoclaved standard custard fillings, the accompanying tables show only figures for the autoclaved standard.

BACTERIOLOGICAL PROCEDURE

Two strains of the organism *Staphylococcus aureus,* which had been isolated from food-poisoning cases attributed to staphylococcal food poisoning, were used throughout this work. Inoculated suspensions of *Staphylococcus aureus* were prepared by adding one c.c. of the organisms from a fresh, 24-hour nutrient broth culture [incubation time, 24 hours at 37°C.(98.6°F.)] to 99 c.c. of sterile distilled water. A known volume of the inoculum was added to each jar of filling or mix and dispersed aseptically. In order to determine the number of organisms added, decimal dilutions of the inoculum were plated out on standard nutrient agar at the time that the fillings were inoculated. The fillings and mixes were incubated for 24 hours at 37°C. and then samples were diluted and plated out on standard nutrient agar. Bacterial counts were made after incubating the agar plates for 48 hours at 37°C.

DISCUSSION OF RESULTS

Commercial Dry-Mixed Puddings: The prepared brands of vanilla, chocolate, and butterscotch made with whole liquid milk supported the growth of *Staphylococcus aureus* (Table 1). When the formulae were modified by substituting water for the milk, the resulting mixes checked bacterial growth but were unsatisfactory from the standpoint of taste.

Vanilla Type Fillings: Since the prepared dry fillings made with water were unsatisfactory, vanilla fillings (No. 2 and No. 3) were prepared according to the formulae previously cited. However, the new vanilla formulae (No. 2 and No. 3) supported the growth of *Staphylococcus aureus* (Table 2); hence, further modifications were made, which consisted of adding sodium propionate and citric acid in varying amounts (Table 2). A maximum of 16 grams of sodium propionate per 3½ pounds of filling was used but showed no inhibitory action. The addition of 3 grams of citric acid to 3½ pounds of filling produced a product (pH 3.43) which was too sour even though it was satisfactory in lowering the growth of *Staphylococcus aureus.* Reductions in the amount of citric acid were made to the point where the taste was satisfactory but when this point was

TABLE 1

Growth of Staphylococcus aureus in Commercial Dry-Mixed Puddings

Type of pudding	Modification	Organisms inoculated per gram of filling; standard plate count 37°C. for 48 hr.	Organisms per gram of filling after 24 hr. incubation at 37°C.; standard plate count 37°C. for 48 hr.		
			Autoclaved pudding	Unautoclaved pudding	Autoclaved standard custard No. 1
Vanilla..............	1 pt. milk per box	80,000	10,000,000	350,000,000	15,000,000
Vanilla..............	1 pt. milk per box	73,000	135,000,000	200,000,000	200,000,000
Butterscotch........	1 pt. milk per box	80,000	30,000,000	30,000,000	15,000,000
Butterscotch........	1 pt. milk per box	73,000	100,000,000	100,000,000	200,000,000
Chocolate............	1 pt. milk per box	73,000	55,000,000	................[1]	200,000,000
Chocolate............	1 pt. milk per box	80,000	3,000,000	15,000,000	15,000,000
Vanilla..............	1 pt. water per box	90,000	6,500	40,000	3,000,000
Vanilla..............	1 pt. water per box	70,000	3,000	6,300	1,000,000
Butterscotch........	1 pt. water per box	90,000	7,000	30,000	3,000,000
Butterscotch........	1 pt. water per box	70,000	2,000	6,000	1,000,000
Chocolate............	1 pt. water per box	90,000	7,500	30,000	3,000,000
Chocolate............	1 pt. water per box	70,000	1,100	15,000	1,000,000

[1] No count; contaminated.

TABLE 3

Growth of Staphylococcus aureus in Pie and Fruit Fillings

Type of filling	Modification	Organisms inoculated per gram of filling; standard plate count 37°C. for 48 hr.	Organisms per gram of filling after 24 hr. incubation at 37°C.; standard plate count 37°C. for 48 hr.		
			Autoclaved filling	Unautoclaved filling	Autoclaved standard custard No. 1
Pumpkin No. 4......	....	82,000	82,000,000	....	100,000,000
Pumpkin No. 4......	....	50,000	100,000,000	196,000,000	200,000,000
Pumpkin No. 4......	....	5,000	10,000,000	....	15,000,000
Pumpkin No. 5......	No milk powder added	82,000	28,000,000	....	100,000,000
Pumpkin No. 5......	No milk powder added	40,000	6,000,000	5,000,000	6,000,000
Pumpkin No. 5......	No milk powder added	5,000	8,250,000	....	15,000,000
Squash No. 7..........	Milk powder added	80,00	15,000,000	6,000,000	3,500,000
Squash No. 7..........	No milk powder added	130,000	205,000,000	162,000,000	300,000,000
Squash No. 7..........	No milk powder added	50,000	490,000,000	640,000,000	200,000,000
Sweet potato No. 6.	Milk powder added (3 oz.)	50,000	530,000,000	610,000,000	200,000,000
Sweet potato No. 6.	No milk powder added	272,000	4,500,000	6,300,000	30,000,000
Raspberry No. 9....	....	80,000	3,000	5,000	3,500,000
Raspberry No. 9....	....	408,000	100	100	2,000,000,00
Peach No. 8...........	....	80,000	40,000	30,000	3,500,000
Peach No. 8...........	....	408,000	200,000	350,000	2,000,000,00

TABLE 2

Growth of Staphylococcus aureus in Vanilla Fillings

Type of filling	Modification	Organisms inoculated per gram of filling; standard plate count 37°C. for 48 hr.	Organisms per gram of filling after 24 hr. incubation at 37°C.; standard plate count 37°C. for 48 hr.			
			Autoclaved filling	pH	Unautoclaved filling	Autoclaved standard custard No. 1
Vanilla No. 2	....	80,000	1,300,000	......	1,500,000	3,500,000
Vanilla No. 2	3 gm. citric acid added per 3½ lb. filling	220,000	5,000	3.43	40,000	47,000,000
Vanilla No. 2	2 gm. sodium propionate added per 3½ lb. filling	220,000	7,000,000	......	69,000,000	47,000,000
Vanilla No. 2	4 gm. sodium propionate added per 3½ lb. filling	220,000	920,000	......	1,600,000	47,000,000
Vanilla No. 3	....	408,000	72,000,000	......	122,000,000	2,000,000,000
Vanilla No. 3	½ gm. citric acid added per 3½ lb. filling	124,000	26,000,000	5.20	35,000,000	15,000,000
Vanilla No. 3	1 gm. citric acid added per 3½ lb. filling	124,000	5,200,000	4.30	6,000,000	15,000,000
Vanilla No. 3	2 gm. citric acid added per 3½ lb. filling	220,000	30,000	3.65	87,000	47,000,000
Vanilla No. 3	3 gm. sodium propionate added per 3½ lb. filling	124,000	76,000,000	......	28,000,000	15,000,000
Vanilla No. 3	8 gm. sodium propionate added per 3½ lb. filling	40,000	5,000,000	......	17,500,000	6,000,000
Vanilla No. 3	16 gm. sodium propionate added per 3½ lb. filling	150,000	350,000,000	......	....	290,000,000
Vanilla No. 3	½ gm. citric acid and 4 gm. sodium propionate added per 3½ lb. filling	124,000	22,000,000	......	30,000,000	15,000,000

Growth of Staphylococcus aureus in Cheese Cake Filling

Type of filling	Modification	Organisms inoculated per gram of filling; standard plate count 37°C. for 48 hr.	Organisms per gram of filling after 24 hr. incubation at 37°C.; standard plate count 37°C. for 48 hr.					Unautoclaved cheese cake filling taste
			Autoclaved sample	pH	Unautoclaved sample	pH	Autoclaved standard custard No. 1	
Cheese cake No. 10......	....	124,000	25,000,000	5.23	....	.....	15,000,000	Satisfactory
Cheese cake No. 10......	....	115,000	3,400,000	5.21	25,000,000	.....	16,000,000	Satisfactory
Cheese cake No. 10......	....	17,000	100,000	5.12	2,000,000	5.32	....	Satisfactory
Cheese cake No. 10......	....	120,000	5,000	4.72	175,000	4.97	....	Satisfactory
Cheese cake No. 10......	1 gm. citric acid added to 500 gm. of filling	40,000	85,000	4.80	....	.....	615,000,000	Slight tartness
Cheese cake No. 10......	1 gm. citric acid added to 500 gm. of filling	44,000	10,000	4.30	10,000	4.41	525,000,000	Very tart
Cheese cake No. 10......	2 gm. citric acid added to 500 gm. of filling	120,000	5,000	3.98	....	4.06	....	Sour
Cheese cake No. 10......	0.60 ml. of 85% lactic acid added to 500 gm. filling	17,000	5,000	4.73	50,000	4.93	....	Satisfactory
Cheese cake No. 10......	1.15 ml. of 85% lactic acid added to 500 gm. filling	17,000	1,500	4.43	115,000	4.83	....	Very slightly tart
Cheese cake No. 10......	1.17 ml. of 85% lactic acid added to 500 gm. filling	120,000	5,000	4.28	5,000	4.42	....	Slight tartness
Cheese cake No. 10......	1.75 ml. of 85% lactic acid added to 500 gm. filling	17,000	500	4.26	1,500	4.67	....	Slight tartness
Cheese cake No. 10......	2.30 ml. of 85% lactic acid added to 500 gm. filling	17,000	5,000	4.23	15,000	4.27	....	Tart
Cheese cake No. 10......	2.34 ml. of 85% lactic acid added to 500 gm. filling	120,000	5,000	4.04	....	4.14	....	Tart

reached (1 gram of citric acid per 3½ pounds of filling, pH 4.30), the resultant filling was not effective in checking bacterial growth. The effect of pH on the growth of *Staphylococcus aureus* is indicated (Table 2).

Pumpkin, Squash, and Sweet-Potato Pie Fillings: The results on the pumpkin, squash, and sweet-potato pie fillings (Table 3) show that all three supported the growth of *Staphylococcus aureus*. The removal of three ounces of powdered milk and three-eighths ounce of butter from Formula No. 4 was not effective in checking the growth of *Staphylococcus aureus* (Table 3).

Peach and Raspberry Fruit Fillings: These fillings (No. 8 and No. 9) were studied for their effect on the growth of *Staphylococcus aureus*. The results as shown (Table 3) indicate that both fruit fillings exerted an inhibitory influence on the growth of *Staphylococcus aureus*.

TABLE 5

Growth of Staphylococcus aureus in Whipping-Cream-Type Mixes

Mix No.	Modification	Organisms inoculated per gram mix; standard plate count 37°C. for 48 hr.	Organisms per gram of mix after 24 hr. incubation at 37°C.; standard plate count 37°C. for 48 hr.			
			Autoclaved mix	pH	Pasteurized mix	pH
11............................	None	100,000	30,000,000	6.22	............[1]	6.35
12............................	None	100,000	33,000,000	6.13	............[1]	6.32
13............................	None	100,000	69,000,000	6.24	............[1]	6.37
14............................	None	100,000	26,000,000	5.85	............[1]	5.97

[1] No count; heavy contamination.

Cheese Cake Filling: A number of these fillings were prepared using Formula No. 10, modified by the addition of varying amounts of citric and lactic acids. We have found baker's cheese to vary in pH from 3.80 to 4.83; however, in the majority of cases the range of pH was between 4.03 and 4.50. Normally, cheese cake fillings prepared without any acid modification have pH values from 5.00 to 5.30. As shown (Table 4) abundant growth of the organism *Staphylococcus aureus* was observed only when the pH of the fillings ranged between 5.21 and 5.32. By increasing the hydrogen-ion concentration of the fillings a retarding of the growth of the organism was noted. The bacteriological counts on cheese cake fillings which had been modified by increasing their acidity showed some variance. It is noteworthy that the organism's growth was gradually suppressed at pH values of 5.12 and lower. The use of lactic acid in place of citric acid was effective in retarding the growth of *Staphylococcus aureus* and did not impart the degree of sourness to the filling as did the citric acid (Table 4).

Whipping-Cream-Type Mixes: The growth of *Staphylococcus aureus* was greatly supported in each autoclaved whipping-cream-type mix. The pH range in all cases was sufficiently high to enhance the growth of this organism. Contamination of heat-resistant organisms in the pasteurized whipping-cream-type mixes masked counts of the organism *Staphylococcus aureus*; bacteriological results are shown (Table 5).

SUMMARY

The commercial dry-mixed puddings containing milk supported the growth of *Staphylococcus aureus*. The growth of this organism was inhibited when water was substituted for milk, but the resulting mix did not produce a satisfactory pudding with respect to taste.

The vanilla fillings, pumpkin, squash, and sweet-potato pie fillings, cheese cake fillings, and whipping-cream mixes were found to support the growth of *Staphylococcus aureus*. Fruit fillings, such as peach and raspberry, contain sufficient acidity to check the growth of this organism.

The addition of citric acid to the vanilla filling in sufficient amounts to lower the pH to 3.43 to 3.65 proved effective in checking the growth of *Staphylococcus aureus* but imparted a sour taste to the product.

An increase in the hydrogen-ion concentration of cheese cake fillings to pH values below 5.12 was effective in gradually retarding the growth of *Staphylococcus aureus*. Lactic acid, when used in place of citric acid, produced a better tasting cheese cake filling and effectively retarded the growth of *Staphylococcus aureus* at pH values between 4.42 and 4.67.

Whipping-cream-type mixes as formulated in this work offer suitable media for the growth of *Staphylococcus aureus*.

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EFFECT OF FERTILIZER TREATMENT ON TOTAL AND
REDUCED ASCORBIC ACID CONTENT OF
POTATO TUBERS[1]

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Investigations on the effect of fertilizer treatment on the ascorbic acid content of fruits and vegetables show considerable variation in the results obtained. A summary of reports appearing in the literature (Table 1) shows an inconsistency in the findings with each of the different treatments employed.

In a study on the effect of fertilizers on the ascorbic acid content of turnip greens produced in four places Reder, Ascham, and Eheart (1943) found that the results varied with locality. Their findings emphasize the importance of environment in determining the nutritive content of the produce. Since in most investigations the number of fertilizers tested was rather limited, an experiment was undertaken in which a total of eight single and mixed fertilizers was used.

The potatoes were produced during the summer of 1945 by the Division of Soils[2] in seven Minnesota localities. Irish Cobbler potatoes were grown at six and Red Warba at one location.

The experiment was set up as a factorial, with three replications at each location. The treatments studied were as follows: untreated (0); superphosphate (43 per cent P_2O_5), applied at the rate of 100 pounds per acre (1P); superphosphate, 200 pounds per acre (2P); muriate of potash (60 per cent), 70 pounds per acre (1K); muriate of potash, 140 pounds per acre (2K); and the following mixed fertilizers: 0-20-20, 200 pounds per acre (PK); 0-20-10, 400 pounds per acre (2PK); 0-20-20, 400 pounds per acre (2P2K); and 4-24-12, 340 pounds per acre (N2PK).

EXPERIMENTAL PROCEDURE

The potatoes were brought in from the field at the time of harvest and were stored in a root cellar until analyzed. The determinations were made between November fifth and December sixth. All samples from a given location were analyzed at the same time. Total ascorbic acid was determined by the Roe and Oesterling (1944) method and reduced ascorbic acid by the modified Morell (1941) procedure. The potato samples were extracted in a Waring blendor, using five per cent metaphosphoric acid; both determinations were made on the same extract. The values presented are the means of duplicate determinations on each of two lots of six

tubers. The analysis of variance technic was employed in testing the significance of the observed differences.

RESULTS AND DISCUSSION

Reduced ascorbic acid values on the moist-weight basis are recorded (Table 2). The means for the tubers from the several locations show considerable variation, the maximum difference occurring between Location II, with a mean of 13.4 milligrams per 100 grams moist weight, and Location VII, with a mean of 9.50 milligrams. For significance at the one-per cent level, a difference of 0.77 milligram is required. Application of this criterion to the data shows that the tubers from some locations contained significantly greater amounts of ascorbic acid than did those from other locations.

TABLE 1

Summary of Studies on Effect of Fertilizers on Reduced Ascorbic Acid Content of Fruits and Vegetables

Authors	Treatment	Food	Effect
Bracewell, Wallace, Zilva (1931)	N	Apples	None
Ijdo (1936)	N	Spinach	Increase
Isgur, Fellers (1937)	N	Swiss chard	Increase
		New Zealand spinach	None
Wynd, Noggle (1945)	N	Oat leaves	Increase
Reder et al. (1943)	N	Turnip greens	None
Reder, Ascham, Eheart (1943)	N	Turnip greens	Decrease
Fellers, Young, Isham, Clague (1934)	NK	Asparagus	None
Illyuviev, Ulanova (1937)	NK	Potato	Increase
Ott (1937)	NP	Potato	Increase
Ijdo (1936)	K	Spinach	Increase
Reder, Ascham, Eheart (1943)	K	Turnip greens	Decrease
Wynd, Noggle (1945)	K	Oat leaves	None
Ott (1937)	K & CaO	Potato	Decrease
Todhunter, Sparling (1938)	P	Peas	None
Wynd, Noggle (1945)	P	Oat leaves	Decrease
Ott (1938)	NPK	Tomato	Decrease
Potter, Overholser (1933)	NPK	Apple	Increase
Sugawara (1938)	NPK	Spinach	Increase
Todhunter (1939)	NPK	Apple	None

A comparison of the means for the tubers from the treated plots with those from the untreated plots yields information as to the effect of the various fertilizers on the reduced ascorbic acid value. For significance at the one-per cent level a difference of 0.87 milligram and at the five-per cent level, 0.65 milligram is needed. It may be observed that of the various fertilizers tested, superphosphate at the higher rate of application increased the ascorbic acid content of the potato tubers; the complete fertilizer and muriate of potash applied at the higher rate decreased the values. The mean ascorbic acid content of the tubers from the untreated plot was 11.37 milligrams per 100 grams moist weight, as compared with a mean of 12.69 milligrams for those from the plots to which superphosphate had been applied at the rate of 200 pounds per acre, a statistically significant increase. The complete fertilizer, 4-24-12, produced a decrease

in reduced ascorbic acid content which was just significant at the five per cent level. Although there was some suppression of the ascorbic acid content when muriate of potash was applied to the soil at the rate of 140 pounds per acre, when tested statistically this decrease was found to be not significant. All other treatments were without effect.

TABLE 2

Effect of Fertilizer Treatments on Reduced Ascorbic Acid Content of Potato Tubers [1]

Treatment	Location							
	I	II	III	IV	V	VI	VII	Mean
0.................................	14.50	11.90	11.25	10.15	11.80	9.20	10.80	11.37
1P...............................	11.50	14.50	11.10	12.40	11.30	9.45	10.20	11.49
2P...............................	13.20	16.25	11.95	13.70	12.25	10.80	10.70	12.69
1K...............................	11.80	13.40	12.35	10.90	10.85	9.20	9.35	11.12
2K...............................	12.45	14.40	9.95	10.70	9.75	9.15	9.85	10.89
PK...............................	13.45	13.00	11.40	13.60	9.50	10.70	9.25	11.56
2PK..............................	13.80	13.00	12.85	9.35	11.40	10.45	8.55	11.34
2P2K............................	12.65	12.50	11.10	14.30	10.25	10.25	8.30	11.34
N2PK............................	14.75	11.65	11.55	8.85	9.40	10.15	8.50	10.69
Mean.........................	13.12	13.40	11.50	11.55	10.72	9.93	9.50	....

	F as calcu- lated	F for significance		Difference for significance	
		5% level	1% level	5% level	1% level
Location......................................	53.20	2.00	2.66	0.58	0.77
Treatment....................................	5.97	2.00	2.66	0.65	0.87
Location × treatment.........................	3.72	2.00	2.66	0.81	1.08

[1] Figures given in milligrams per 100 grams moist weight.

The interaction between locations and treatments is also significant and indicates that the influence of the treatment varied with the location. This may be illustrated by citing the effect of superphosphate at Locations I and II. At the former the tubers from the untreated plot averaged 14.50 milligrams; with treatment 1P, 11.50 milligrams; and with 2P, 13.20 milligrams. At Location II, on the other hand, the mean of the values for the untreated plot was 11.90 milligrams; for treatment 1P, 14.50 milligrams, and for 2P, 16.25 milligrams. From this it is apparent that the application of superphosphate greatly benefited the tubers produced at Location II but not those at Location I.

Since considerable variation in moisture content was observed in the tubers produced at the several locations, it seemed desirable to determine whether the findings would be altered by computation of the data on the dry-weight basis. The data presented (Table 3) show that both location and treatment caused highly significant differences in ascorbic acid values. Of the fertilizers tested, superphosphate applied at the rate of 200 pounds per acre increased the ascorbic acid content significantly, the mean for the untreated plot being 46.6 milligrams and for the treated plot 51.5 milligrams per 100 grams dry weight. The suppressing effect of the complete fertilizer noted on the moist basis, however, just failed of significance at the five-per cent level.

Total ascorbic acid values for the potato tubers are shown (Tables 4. and 5). A comparison of the data in these tables with the data in Tables 2 and 3 shows that the total ascorbic acid content was considerably greater than the reduced ascorbic acid, indicating that appreciable amounts of dehydroascorbic acid had been formed in the interval between harvest and analysis. This is in accord with the observation of Smith and Gillies (1940) who found that the dehydro form of ascorbic acid increases fairly rapidly during storage.

TABLE 3

Effect of Fertilizer Treatments on Reduced Ascorbic Acid Content of Potato Tubers [1]

Treatment	Location							
	I	II	III	IV	V	VI	VII	Mean
O	56.4	43.2	44.0	39.6	47.2	49.2	46.8	46.6
1P	47.4	53.6	42.3	49.1	45.3	43.5	42.8	46.3
2P	51.8	65.6	45.0	53.7	52.6	48.8	43.4	51.5
1K	48.4	52.8	50.3	44.8	51.6	44.4	43.7	48.0
2K	52.8	57.0	44.4	45.2	42.2	44.2	45.8	47.4
PK	55.2	49.2	45.2	54.7	43.1	50.4	38.9	48.1
2PK	57.1	47.4	48.6	38.6	47.0	49.6	37.6	46.6
2P2K	52.2	46.6	45.0	58.8	41.6	50.3	37.0	47.4
N2PK	59.0	46.4	46.4	36.2	43.2	47.2	33.1	44.5
Mean	53.4	51.3	45.7	46.7	46.0	47.5	41.0	.....

	F as calculated	F for significance		Difference for significance	
		5% level	1% level	5% level	1% level
Location	23.72	2.00	2.66	2.33	3.10
Treatment	4.15	2.00	2.66	2.65	3.52
Location × treatment	4.07	2.00	2.66	3.30	4.39

[1] Figures given in milligrams per 100 grams dry weight.

As in the case of reduced ascorbic acid, locality influenced markedly the total ascorbic acid content of the potato tubers, close correspondence being noted in the two sets of values in nearly every instance. On the moist-weight basis, differences in total ascorbic acid of 0.69 and 0.91 milligram were found to be significant at the five- and one-per cent levels, respectively.

The treatment means given (Table 4) show that application of superphosphate at the rate of 200 pounds per acre augmented the total ascorbic acid content of the potato tubers but not to a significant degree. A depressing effect was noted as a result of the application of muriate of potash at the rate of 140 pounds per acre, of 0-20-20 at the rate of 400 pounds per acre, and a complete fertilizer, 4-24-12, at the rate of 340 pounds per acre. The latter had the most marked effect.

The fact that the interaction, location × treatment, was highly significant shows that the tubers responded to the treatments in a differential manner at the several locations. By way of illustration it may be pointed out that the complete fertilizer had no inhibiting effect at Locations I and III but did at the remaining five locations.

TABLE 4

*Effect of Fertilizer Treatments on Total Ascorbic Acid Content of
Potato Tubers*[1]

Treatment	Location							
	I	II	III	IV	V	VI	VII	Mean
O	18.95	19.25	16.50	14.70	17.35	14.55	15.50	16.69
1P	20.05	18.90	16.50	14.15	17.15	14.45	14.35	16.51
2P	18.25	21.15	18.80	14.75	17.15	15.40	14.75	17.18
1K	17.40	18.65	17.90	15.20	15.35	14.20	14.40	16.16
2K	16.60	19.30	15.05	15.30	15.60	13.85	14.35	15.72
PK	17.10	19.10	18.30	14.40	13.90	15.55	13.25	15.94
2PK	18.45	17.60	19.35	13.90	16.40	14.45	12.45	16.09
2P2K	17.15	17.70	16.60	14.95	16.55	14.00	13.35	15.76
N2PK	19.00	15.25	17.35	12.95	13.90	13.80	12.35	14.94
Mean	18.11	18.54	17.37	14.48	15.93	14.47	13.86	......

	F as calculated	F for significance		Difference for significance	
		5% level	1% level	5% level	1% level
Location	62.24	2.00	2.66	0.69	0.91
Treatment	5.46	2.00	2.66	0.78	1.03
Location × treatment	2.10	2.00	2.66	0.97	1.29

[1] Figures given in milligrams per 100 grams moist weight.

TABLE 5

*Effect of Fertilizer Treatments on Total Ascorbic Acid Content of
Potato Tubers*[1]

Treatment	Location							
	1	II	III	IV	V	VI	VII	Mean
O	73.6	70.0	64.8	57.5	69.4	78.0	67.2	68.6
1P	78.7	69.8	62.7	55.9	68.8	66.8	60.4	66.2
2P	76.0	85.3	71.0	57.6	73.8	69.6	60.0	70.4
1K	71.2	73.3	73.0	62.7	72.7	68.6	67.3	69.8
2K	70.4	76.4	67.2	64.4	67.4	66.6	66.7	68.4
PK	76.5	72.2	72.2	58.0	62.9	72.9	55.6	67.2
2PK	76.3	64.2	73.4	57.4	67.5	68.6	55.0	66.0
2P2K	70.6	66.0	67.2	61.6	67.5	68.5	59.6	65.9
N2PK	76.0	60.6	69.8	53.2	63.8	64.0	48.2	62.2
Mean	74.4	70.9	69.0	58.7	68.2	69.3	60.0	......

	F as calculated	F for significance		Difference for significance	
		5% level	1% level	5% level	1% level
Location	30.85	2.00	2.66	2.93	3.89
Treatment	4.53	2.00	2.66	3.32	4.41
Location × treatment	2.00	2.00	2.66	4.14	5.50

[1] Figures given in milligrams per 100 grams dry weight.

The data computed on the dry-weight basis show the augmenting effect of the superphosphate noted on the moist-weight basis, but the increase in this instance is not statistically significant. The inhibiting effect of the complete fertilizer is very marked, but the effect of 0-20-20, while still evident, just fails of significance. The inhibiting effect of muriate of potash noted on the moist basis is not evident on the dry basis, from which it is apparent that these tubers were low in dry matter.

The findings in the present study help to clarify the inconsistencies noted in the literature on the effect of fertilizer treatment on the ascorbic acid content of vegetables, in that the treatment effect varied with the locality. For example, although the over-all effect of the superphosphate was to augment the ascorbic acid content, as indicated by statistical analysis of the data, actually increases occurred in the tubers from only five of the locations. Of the remaining two places, the tubers from one showed no change, those from the other, a decrease. Similarly, the statistically significant decrease in ascorbic acid associated with the application of a complete fertilizer is attributable to the marked reduction noted in the tubers from three of the locations, the tubers from three locations showing no essential change and those from one, a significant increase.

SUMMARY

The effect of fertilizer treatments on the reduced and total ascorbic acid content of potato tubers grown in seven Minnesota localities was investigated. The treatments used and the rates of application were as follows: untreated; superphosphate, 100 pounds per acre; superphosphate, 200 pounds per acre; muriate of potash, 70 pounds per acre; muriate of potash, 140 pounds per acre; and the following combined fertilizers: 0-20-20, 200 pounds per acre; 0-20-10, 400 pounds per acre; 0-20-20, 400 pounds per acre; 4-24-12, 340 pounds per acre.

Superphosphate at the higher rate of application produced a statistically significant increase in the reduced and total ascorbic acid content on both the moist- and dry-weight bases.

Muriate of potash at the higher level of application tended to decrease the reduced and total ascorbic acid content on the moist-weight basis, but not on the dry-weight basis.

The complete fertilizer tended to suppress the reduced and total ascorbic acid content on both the moist- and dry-weight bases.

The effect of the fertilizer treatment varied with the location.

Environmental factors associated with the location at which the potatoes were produced were of greater importance in determining the ascorbic acid content of the tubers than were the fertilizers tested.

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VARIATIONS IN COMPOSITION OF FRANKFURTERS WITH SPECIAL REFERENCE TO COOKING CHANGES

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A considerable amount of literature exists on the composition of frankfurters and on the changes that frankfurters undergo during processing. Very little information is available, however, as to the changes in composition that occur when frankfurters are recooked for consumer use. While the frankfurter, when purchased, is a cooked meat product and is sold in a form ready for consumer use, the usual practice is to heat frankfurters before using. Heating for a short period of time in water that has been brought to a boil is a recommended procedure. Since the cooked frankfurter is the product that is ultimately consumed, it seemed desirable to investigate the changes in chemical composition of frankfurters with cooking. This report is concerned with the changes in moisture, protein, fat, and ash content of frankfurters with cooking. Six- to 10-pound samples of first grade ''skinless'' and animal-casing frankfurters were purchased from 10 Chicago packing houses. Two types of cooking were used: a mild type of cooking described as the ''consumer cook,'' and a ''severe cook'' that represented the extreme in the cooking of frankfurters.

EXPERIMENTAL PROCEDURE

The consumer cook was performed in the following manner: To 500 ml. of boiling tap water, 400 to 450 grams of frankfurters were added. Heating was continued until the water boiled gently; this usually required about five minutes. The container of frankfurters was removed from the heater and allowed to cool to 60°C.(140°F.). Fifteen to 20 minutes were usually required for this cooling. The cooking water was drained from the frankfurters, weighed, and samples withdrawn for solids, ash, protein, and carbohydrate. The cooked frankfurters were placed in tared Mason jars and weighed after cooling to room temperature. The entire sample was passed twice through a small food chopper and the finely ground material was returned immediately to the Mason jar. Samples were withdrawn for moisture, protein, and ash determinations. The dry residue from the moisture analysis was used for fat analysis.

The severe cook was performed by boiling a liter of tap water, adding a 400- to 450-gram sample of frankfurters, and continuing the boiling for 30 minutes. The container was removed from the heater and allowed to cool to 60°C. The cooking water and cooked frankfurters were treated as described for the consumer cook procedure given above.

The frankfurter samples, on drying, break down and the emulsified fat forms a layer of grease in the weighing bottle. The nonfat portion of the frankfurter solids is chiefly protein and, on drying, this protein forms a crusty residue from which most of the fat is rendered. Uniform

sampling of the dried frankfurter is almost impossible because of this rendering of the fat. As a result, moisture, protein, and ash were determined on separate samples of the moist frankfurters. Relatively large samples and rapid weighing on a torsion balance gave excellent results.

Moisture: Samples of 25 to 30 grams of the ground moist frankfurter were transferred to tared aluminum weighing dishes. The weighed samples were placed in a vacuum oven at 60°C. and evacuated for 24 hours. An additional 16 hours' evacuation gave values of less than 0.1 per cent difference in moisture content.

Fat: The dry residue from the moisture determination was transferred to an extraction thimble. The separated fat in the bottom of the weighing dish was transferred to the thimble with small portions of Skelly-Solve "F." The fat was extracted in a Soxhlet apparatus for 16 hours using Skelly-Solve "F." The solvent was removed by boiling and the fat was dried to a constant weight of 105°C.(221°F.). The fat-free residue was weighed and the fat calculated by difference. This procedure is an excellent method for checking the direct fat determination.

Ash: Samples of six to eight grams of ground moist frankfurters were weighed in tared porcelain crucibles. The samples were dried in an oven at 105°C. and finally transferred to the muffle furnace. After heating at a dull red heat for approximately four hours, the gray residue was weighed and the weight recorded as ash.

Protein: Samples of eight to 10 grams of ground moist frankfurters were transferred to 800-c.c. Kjeldahl flasks. Fifteen grams of potassium sulfate, 0.4 gram of copper wire, and 65 c.c. of concentrated sulfuric acid were added to the flask and the mixture heated for four hours. The clear digestate was diluted with 300 c.c. of distilled water and cooled. Sodium hydroxide solution was added cautiously so a good layering of the alkali resulted; 120 c.c. of the 50-per cent caustic was needed to liberate the ammonia. Mossy zinc was added and the ammonia was distilled into two per cent boric acid solution. The ammonia was titrated with 0.5 N hydrochloric acid using brom cresol green as an indicator. The factor 6.25 was used for converting nitrogen values to protein.

Cooking Water Analysis: An aliquot of the cooking water was evaporated to dryness in a tared porcelain crucible and the residue was dried in the vacuum oven to a constant weight. The residue was then ashed at dull red heat in the muffle furnace. The resulting gray white residue was weighed and the weight recorded as ash. An aliquot of the cooking water was used for nitrogen analysis. Fifteen grams of potassium sulfate, 0.4 gram of copper wire, and 25 c.c. of concentrated sulfuric acid were used. Duplicate samples of the cooking water were used for carbohydrates before and after inversion. The copper reduction method of Munson and Walker was used, A.O.A.C. (1940). The carbohydrate values reported (Tables 4 and 5) are expressed as glucose equivalents.

DISCUSSION

An excellent report on the composition of frankfurters and other sausage materials is given by Hoagland (1932). He reported variations in frankfurter composition of the same general magnitude as our analysis

for the skinless and animal-casing frankfurter. Pahlke (1944) reported an analysis of frankfurter emulsion, frankfurters processed from the emulsion using animal and cellulose casings, and the same frankfurters after cooking. Solids, protein, calcium, and phosphorus were determined on samples of the above laboratory preparations and no significant differences were obtained between frankfurters processed in animal and cellulose casings.

Our samples were obtained from the various packing houses without any knowledge of their past history. The frankfurters were both of the skinless and animal-casing type and were identical with frankfurters sold for consumer purposes. "Skinless" frankfurters are prepared by stuffing the frankfurter emulsion into cellulose tubes. The frankfurter is processed, and after processing, the cellulose tube is stripped from the finished product. "Animal-casing" frankfurters are prepared by stuffing frankfurter emulsion into tubes obtained from animal sources. The casings are usually processed from sheep, hog, and calf intestines; the animal-casing frankfurters in this report were processed in sheep casing. This type of frankfurter retains the casing as an integral part of the finished product.

TABLE 1

Composition of Purchased Frankfurters

Packing Co.	Number of trials	Moisture		Protein		Ash		Fat	
		Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal
		pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.
A	3	56.62	57.84	13.50	14.16	2.11	2.11	24.42	22.55
B	3	65.41	60.08	14.47	15.70	2.65	3.48	14.20	18.04
C	3	52.50	55.70	13.31	13.35	2.11	1.99	28.75	26.09
D	3	61.84	61.44	12.08	12.56	2.08	2.75	21.60	20.54
E	3	61.68	58.71	15.31	14.85	1.80	1.35	18.23	22.83
F	2	47.55	54.16	10.52	10.88	1.22	1.39	35.79	26.51
G	2	57.61	58.05	14.30	14.46	2.08	1.94	24.50	24.21
H	2	58.22	58.00	12.97	13.14	1.60	2.00	19.04	20.68
I	2	58.60	58.02	12.08	10.81	2.28	1.88	20.26	22.19
J	2	59.61	60.99	13.89	14.26	3.05	3.58	17.12	14.84
Average		57.96	58.29	13.24	13.42	2.09	2.25	22.39	21.85

A rather wide variation in composition of frankfurters from the different packing houses is shown (Table 1). In some samples a wide variation occurred between animal-casing and skinless frankfurters from the same source. Nevertheless, a close agreement occurred on the averaging of the percentages of constituents determined on the skinless and animal-casing frankfurters. In no pair of analyses from a single source was the variation between skinless and animal-casing frankfurters as great as the variation between the composition of the same type of frankfurter from the other sources. For the consumer's interest, Table 1 represents what he buys, Tables 2 and 3 give the composition of what he eats, and Tables 4 and 5 show the composition of what the consumer discards. The "consumer-cooked" frankfurters retained an average of over 96 per cent of their solids, whereas the "severe-cooked" frankfurters retained an average of 91 per cent of their solids. However, the severe-cook procedure is rarely

TABLE 2

Composition of "Consumer-Cooked" Frankfurters

Packing Co.	Number of trials	Change		Moisture		Protein		Ash		Fat	
		Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal
		pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.
A............	3	+1.00	+1.56	59.04	59.44	13.38	12.82	2.51	2.39	21.92	22.35
B............	3	−14.30	−7.70	63.39	60.65	15.80	16.40	1.79	1.54	15.72	18.35
C............	3	+3.16	+2.49	55.85	57.01	12.35	12.59	1.19	1.35	28.00	26.96
D............	3	+0.64	+1.12	64.17	64.00	11.90	12.62	1.48	1.91	21.36	19.48
E............	3	+1.19	−7.35	64.98	59.85	15.25	14.44	1.58	0.67	17.46	22.48
F............	2	+1.72	+2.07	50.64	57.88	10.24	11.84	0.72	1.04	34.58	23.62
G............	2	+1.64	+2.00	59.39	59.72	14.08	13.88	1.60	1.51	23.71	23.40
H............	2	+3.04	+3.49	61.42	59.54	12.55	12.82	2.46	2.41	18.36	18.99
I............	2	+2.09	+2.57	60.74	59.00	12.16	10.60	1.77	1.47	18.86	19.92
J............	2	+6.21	+5.01	63.66	63.66	13.40	13.80	2.72	2.76	14.84	14.84
Average........		+0.64	+0.53	60.33	60.08	13.11	13.18	1.78	1.70	21.48	21.04

TABLE 3

Composition of "Severe-Cooked" Frankfurters

Packing Co.	Number of trials	Change		Moisture		Protein		Ash		Fat	
		Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal
		pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.
A............	3	+1.39	−2.12	58.30	58.19	13.05	13.46	2.74	2.53	23.17	23.37
B............	3	−17.30	−29.40	63.35	58.84	16.06	20.30	0.83	2.04	15.47	15.76
C............	3	+5.52	−4.79	56.90	57.12	11.66	11.99	1.18	0.74	27.60	27.90
D............	3	+0.76	−3.58	64.60	64.54	11.71	12.75	1.34	1.74	21.16	19.96
E............	3	+4.01	+1.32	65.20	63.60	14.34	13.92	0.76	0.52	15.23	20.58
F............	2	+3.30	−0.24	52.34	59.41	10.06	11.13	0.59	0.44	33.94	25.29
G............	2	+10.90	−1.76	59.88	60.32	14.12	14.12	1.28	1.03	23.92	23.98
H............	2	−2.12	−9.23	61.74	60.10	12.62	13.88	1.69	0.90	18.93	19.26
I............	2	+2.84	−8.58	63.50	59.98	11.48	11.56	1.08	0.51	17.30	22.17
J............	2	+6.78	−4.95	66.90	67.10	12.60	14.21	2.05	2.16	11.89	11.90
Average.......		+1.11	−6.51	61.27	60.91	12.77	13.73	1.35	1.26	20.86	21.02

TABLE 4

Percentages of "Consumer-Cook" Water Constituents Based on Original Weight of Purchased Frankfurters

Packing Co.	Number of trials	Solids		Ash		Protein		Carbohydrates		Carbohydrates after inversion	
		Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal
		pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.
A	2	1.03	1.13	0.52	0.71	.23	.21	.07	.08	0.14	0.17
B	2	2.45	2.48	1.09	1.29	.64	.60	.44	.52	1.36	1.21
C	2	1.01	0.93	0.62	0.54	.20	.20	.11	.13	0.30	0.14
D	2	0.98	1.04	0.53	0.55	.20	.20	.16	.17	0.24	0.26
E	2	0.75	0.81	0.49	0.54	.22	.19	.12	.17	0.19	0.23
F	2	1.12	1.29	0.95	0.62	.21	.24	...	...	...	...
G	2	0.78	0.84	0.51	0.54	.20	.21	.09	.08	0.18	0.16
H	2	1.80	1.59	0.69	0.64	.23	.22	.53	.45	0.64	0.60
I	2	1.12	0.85	0.57	0.51	.22	.13	.18	.10	0.39	0.33
J	2	1.72	1.50	0.80	0.76	.25	.23	.43	.40	1.02	1.29
Average		1.28	1.25	0.68	0.67	.26	.24	.24	.23	0.50	0.49

TABLE 5

Percentages of "Severe-Cook" Water Constituents Based on Original Weight of Purchased Frankfurters

Packing Co.	Number of trials	Solids		Ash		Protein		Carbohydrates		Carbohydrates after inversion	
		Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal	Skinless	Animal
		pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.
A	2	1.35	1.86	0.72	0.90	.16	0.19	0.17	0.24	0.21	0.34
B	2	3.75	4.76	1.53	2.19	.43	0.64	1.01	0.96	2.26	1.98
C	2	2.09	3.49	1.07	1.59	.50	0.92	0.21	0.69	0.23	0.77
D	2	2.01	2.95	0.85	1.60	.51	0.65	0.58	0.86	0.67	1.01
E	2	1.63	2.23	1.03	1.32	.41	0.72	0.43	0.74	0.52	0.99
F	2	2.23	3.82	1.01	1.42	.44	0.86	...	...	...	...
G	2	1.57	2.16	0.92	1.24	.46	0.69	0.29	0.32	0.49	0.66
H	2	4.53	5.19	1.35	1.45	.80	1.10	1.36	1.62	2.24	2.64
I	2	2.22	4.25	1.02	1.67	.44	0.82	0.36	0.51	0.81	1.19
J	2	4.06	5.57	1.46	2.13	.69	1.12	1.11	1.03	3.23	2.86
Average		2.54	3.63	1.10	1.55	.48	0.77	0.61	0.77	1.18	1.38

approached in the preparation of frankfurters for food purposes. The column labeled "per cent of change" (Tables 2 and 3) shows the percentage changes in weight of the cooked frankfurters calculated in terms of the initial weight of the uncooked frankfurters. When a loss in weight occurred, the percentage was recorded with a minus sign to denote shrinkage. A plus sign denotes a percentage gain in weight. Frankfurters that showed high shrinkage values gave a much larger proportion of their solids to the cooking water than frankfurters that showed little or no shrinkage.

SUMMARY AND CONCLUSION

A wide variation exists in the composition of frankfurters sold for consumer use. This variation exists in both skinless and animal-casing frankfurters. Frankfurters lose very little of their solid content when recooked for consumer use. A large portion of the frankfurter remains intact even after drastic boiling procedures. Both skinless and animal-casing frankfurters behaved in a similar manner when subjected to the consumer and severe cooking procedures.

ACKNOWLEDGMENT

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RELATION OF TENDERNESS OF BEEF TO AGING
TIME AT 33-35°F.

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Since the consumer demands tenderness in meat, study of beef tenderization has been a major project in our research program for several years. From time to time what seemed to be paradoxical results were obtained. Such results could be duplicated and apparently were beyond the errors expected by the testing technique which is described elsewhere by Deatherage and Reiman (1946). These apparent discrepancies were, for the most part, results contrary to the premise that tenderness increases with post-mortem age of meat. If meat were a homogeneous substance one might expect that tenderness would vary directly with age, but some reflection on the heterogeneous structure of meat indicated that perhaps beef does not tenderize regularly with increasing aging time.

It is impossible in this paper to review the many contributions to the knowledge of tenderness in meat. The works of Moran and Smith (1929 *et seq.*), Smorodintsev and co-workers (1935 *et seq.*), Satorius and Child (1938), Steiner (1939), Winkler (1939), Griswold and Wharton (1941), Paul, Lowe, and McClurg (1944), and Ramsbottom, Strandine, and Koonz 1945) are particularly relevant to this investigation. Up to this time, however, there has been little information available on actual tenderness changes during the process of aging meat. The lack of this information might have been due to some extent to the variability in results obtained from different tenderness-testing techniques. Since, in this laboratory, a reproducible tenderness-tasting technique has been devised and since its reliability has been evaluated to some degree, the authors felt that they might be able to estimate the relation of tenderness to post-mortem age of beef. Such information would serve a twofold purpose: (1) supply information essential for a better understanding of the nature of tenderness in meat and (2) give data concerning the efficiency of tenderizing beef commercially by aging at 0.6-1.7°C.(33-35°F.). Accordingly this study was made of the relation between tenderness of beef and post-mortem age of beef.

EXPERIMENTAL PROCEDURE

Two lots of commercially slaughtered cattle were used in this investigation. The first lot consisted of 10 animals (Nos. 81, 82, 86, 87, 88, 89, 90, 95, 96, and 97). These were slaughtered the same day and their carcasses graded U. S. Good, except Nos. 86, 88, and 96 which graded U. S. Commercial. After 24 hours, both full loins from each carcass were excised and put into the 33-35°F. cooler and tested 2, 6, 10, 17, 24, 31, and 38 days

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after slaughter. Two weeks after the first lot was started the second lot of four cattle was slaughtered (Nos. 1, 2, 3, and 4). Of this lot No. 1 graded U. S. Good, while the remaining three graded U. S. Choice. These four carcasses were treated as in the first lot and tested 3, 6, 10, 17, 24, 31, and 41 days after slaughter. The test meat in some instances was used up before the last test was done. Test meat was cut from one of the two loins until the whole loin was used up before cutting the second loin. For one half of the carcasses the right loin was used first, while in the remaining carcasses the left loin was used first. Four test steaks, seven-eighths inch thick, were cut from the excised loins for each test. This permitted duplicate taste testing by six experienced judges. The test steaks were cut from anterior end to posterior end for one half of the loins and in reverse order for the other half. Before taking the test steaks, one-quarter inch was cut off from the end of the loin and discarded. The steaks were broiled and tested as described in this journal by Deatherage and Reiman (1946) with the change that no preference ratings were made, since the two samples presented to the judges each time represented two different animals. The judges rated the broiled segments of the longissimus dorsi muscle according to the tenderness scale of 10, very tender; 8, tender; 6, slightly tough; 4, tough; and 2, very tough. With only minor substitutions the same panel of six experienced judges was used throughout the study.

At the end of each test period the tenderness level of each carcass was computed by averaging the 12 individual scores given by the six judges in the duplicate testing of the loins. This tenderness value, therefore, corresponds to the tenderness level obtained in the duplicate half tests described in the above-mentioned paper.

RESULTS AND DISCUSSION

The reproducibility of results obtained in this study was comparable to, or somewhat better than, that previously reported. The data of this study were equivalent to 93 duplicate half tests, and the standard and probable deviations for the single half test by six judges were found to be 0.61 and 0.40, respectively. In the previous report these figures based on data from 438 duplicate half tests were 0.73 and 0.49, respectively. These deviations are expressed on the per-judge basis rather than on the panel or six-judge basis. The smaller deviations found in this study might result from using the same panel of tasters throughout. The results of this investigation are given (Table 1).

Each value represents the average of 12 scores in duplicate tests by six judges. The initial tenderness levels (two or three days) of the test animals fall into two groups. Group I consists of 12 animals with initial tenderness levels in the range of 3.08 to 5.17, a fairly close grouping permitting some degree of critical analysis. Group II consists of only two animals of high initial tenderness, 7.33 and 7.75. These animals were quite young, 18 months old or less. Since only two carcasses fall into Group II, we can report only the results as any conclusions drawn from these animals would be too speculative. However, since these animals of high initial tenderness seem to present quite a different picture when compared with Group I animals, some useful information of basic interest might result from more complete study of animals falling into this category.

The tenderness curves for each animal are shown (Figs. 1, 2, and 3) for the animals of Group I, and (Fig. 4) for those of Group II. It is at once apparent that not all the carcasses increase in tenderness with age throughout the period of observation. The decrease in tenderness during the aging period in some carcasses appears to be quite real as the data are outside the realm of experimental error.

TABLE 1

Relation of Tenderness to Post-Mortem Age of Beef

Animal No.	Days after slaughter								
	2	3	6	10	17	24	31	38	41
81................	3.08	......	4.33	5.42	6.92	6.25	8.00	8.08	......
82................	4.58	......	4.75	5.92	7.17	7.42	8.25	7.50	......
86................	3.42	......	4.83	5.92	5.92	5.83	6.75	7.17	......
87................	3.67	......	4.08	5.50	5.42	5.92	6.17	......	......
88................	4.75	......	5.67	5.75	7.00	7.83	7.58	7.83	......
89................	5.00	......	5.67	6.08	7.17	7.08	7.92	8.17	......
90................	5.17	......	6.42	7.08	7.42	6.00	7.83	8.00	......
95................	4.58	......	6.42	7.17	7.58	7.83	9.00	......	......
96................	4.42	......	4.58	5.17	6.33	7.08	7.75	7.83	......
97................	4.08	......	5.58	7.08	7.67	9.00	8.50	8.50	......
1................	......	4.00	5.33	5.92	7.00	6.75	7.00	......	......
2................	......	7.75	7.67	7.00	7.92	8.50	7.25	......	7.67
3................	......	7.33	7.47	8.67	8.17	8.75	8.50	......	......
4................	......	4.08	5.58	5.75	7.25	5.17	6.58	......	......

In Group I some animals appear to become progressively more tender throughout the aging period, while others definitely seem to become tougher at some time during the aging. Individual variations in animals account for these differences, of course, but since the 12 animals of this group were initially close together in tenderness, it was thought that the results would permit some analysis as a collective unit. Accordingly, the average and median tenderness values have been computed for the 12 animals whose tenderization was followed for 31 days and for the eight animals followed for 38 days; these data are given (Table 2 and Fig. 5). It should be pointed out that since only two of the 12 animals were initially tested at

TABLE 2

Changes in Average and Medium Tenderness Values During Post-Mortem Aging at 33-35° F.

Post-mortem age at 33-35°F.	8 animals		12 animals	
	Average	Median	Average	Median
days				
2..	4.33	4.50	4.24	4.25
6..	5.21	5.20	5.26	5.42
10..	6.05	5.92	6.06	5.92
17..	6.95	7.08	6.90	7.08
24..	7.06	7.07	6.84	6.91
31..	7.82	7.88	7.61	7.79
38..	7.88	7.90	......	......
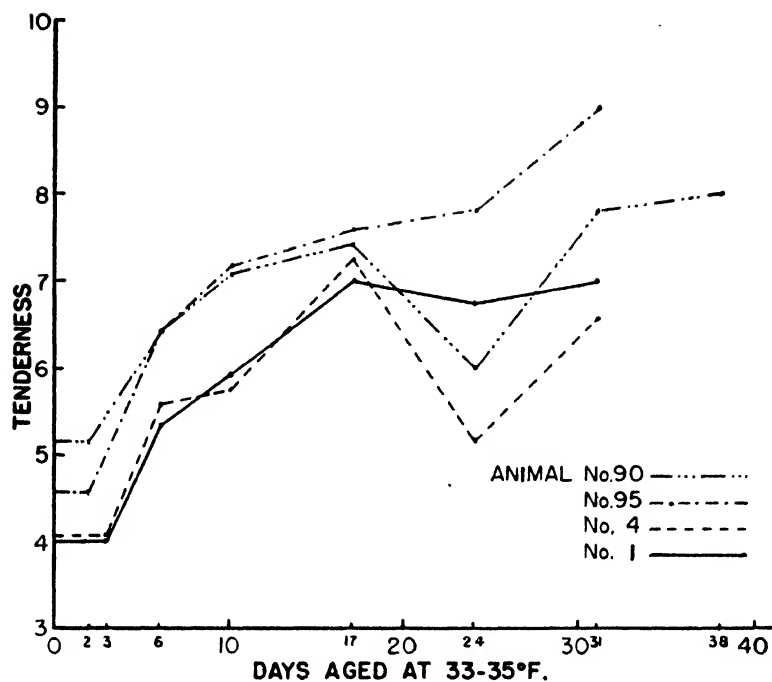
FIG. 1
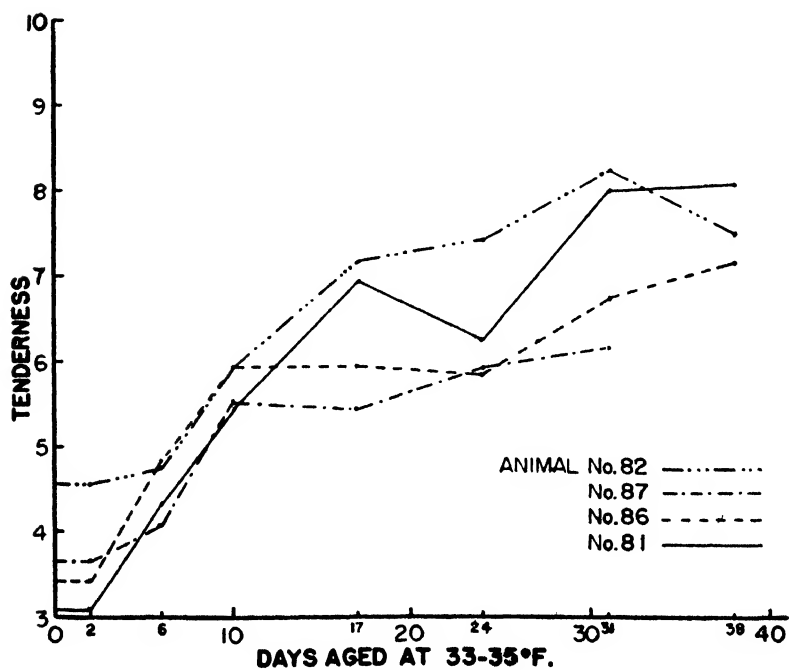
FIG. 2

three days' rather than two days' post-mortem, these two values were included in the average and median values computed for the two days' aging.

Figure 5 clearly demonstrates that tenderness is not a smoothly increasing function with age of the carcass held at 33-35°F. Group I, as a whole, shows a break in tenderization at about 17 days, at which time there is a slight drop in tenderness from 17 to 24 days' aging. At 31 days there appears to be some improvement beyond the 17- and 24-day levels. That there is a plateau or drop in the tenderization of the beef under study there can be little doubt. Since tenderness is not a smoothly increasing function with age, it is reasonable to suppose that more than one major factor (or structure) of beef contributes to the attribute of quality the consumer calls tenderness. Further, it may be considered that individual differences in animals (Figs. 1, 2, and 3) indicate the relative variation with respect to each other of these major factors responsible for toughness. It was considered that perhaps the irregularity of the curves (Fig. 5) might result from using both loins of the test animals or from using test meat from end to end of the longissimus dorsi muscle throughout this study. The authors are inclined to discount these arguments in view of the work of Ramsbottom *et al.* (1945) and from the fact that tenderness differences have not been observed from side to side of the animal as long as comparable muscles were used for testing, nor has the observation been made that there were differences in tenderness in the longissimus dorsi muscle from the same animal.

At this time it is only possible to speculate as to the underlying causes for the observations made in this investigation. Numerous authors have studied the role of connective tissue (both collagenous and elastic) in beef tenderness, while others have noticed changes in muscle fibers. Without reviewing the contributions of others the foregoing results may be interpreted by reflection on the work of these authors, particularly Moran and Smith (1929), Smith (1938), Steiner (1939), Griswold and Wharton (1941), Paul, Lowe, and McClurg (1944), and Ramsbottom, Strandine, and Koonz (1945). In the fresh uncooked state, meat consists of connective tissue fibers (both collagenous and elastic) holding together muscle fibers which in themselves may be roughly considered to be thin sheaths (sarcolemmae) enclosing a fluid substance which is coagulable on heating. More generally, muscle may be thought to consist of connective tissue (or preformed fibers) and intracellular fluid which is made up largely of coagulable protein in some sort of aqueous system. On cooking, this intracellular fluid coagulates to form a fiber; thus, cooked meat may be thought to consist of two fibrous structures—connective tissue or preformed fibers (C) and coagulated muscle plasma (M).

Before going further in this discussion it should be pointed out that chewing of meat may be considered a composite of one or more of several functions, such as shearing, tearing, crushing, grinding, and cutting, and that the jaws of the taster have a limited capacity to chew the meat and in this respect differ from a mechanical device to shear or crush the meat. As this limit is approached, meat is said to be tough; when meat is called tender, relatively little work is done in chewing. Thus tender meat puts
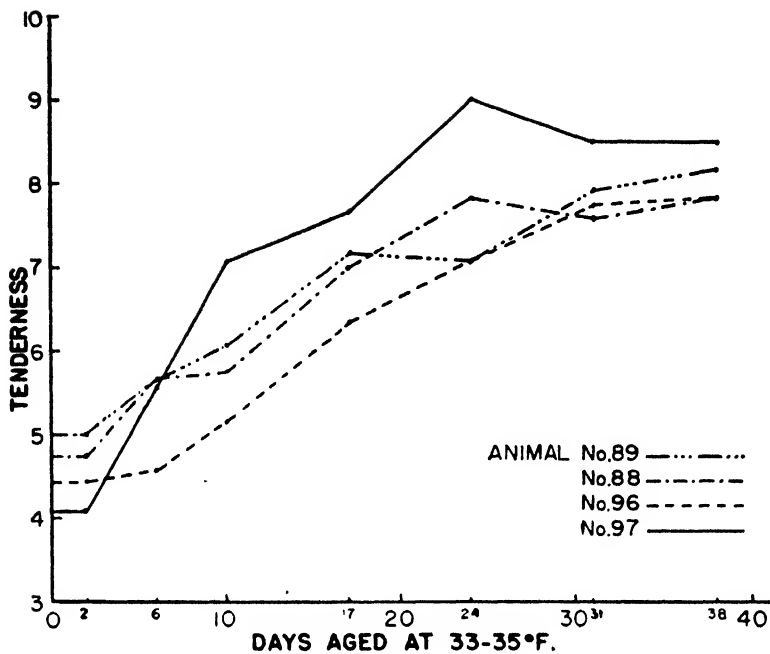
Fig. 3
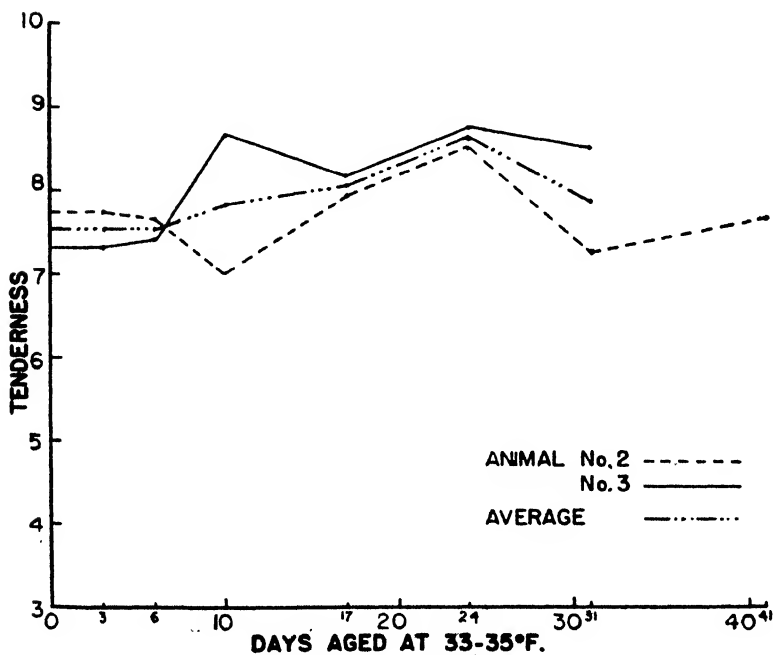
Fig. 4

relatively little strain on the jaw of the taster, whereas tough meat puts on the jaw a strain which approaches the maximum of the jaw muscle.

Returning to the concept that cooked meat consists of fibers C and M, it has been observed that before cooking and during the aging process the precursors to M seem to disintegrate at a faster rate than C [Paul, Lowe, and McClurg (1944) and the authors' unpublished observations]. If this be the case it is not difficult to visualize that from the taster's viewpoint toughness owing to C is changed little compared with that owing to M. As aging proceeds toughness owing to M might tend to approach 0, while that owing to C is unchanged. If this condition remains for a period of time, then the taster observes no tenderization during this period. Aging further, C might disintegrate to the point that the fibers begin to lose their strength from the taster's point of view, at which time toughness owing to C diminishes. Such an approach could account for a plateau (Fig. 5).
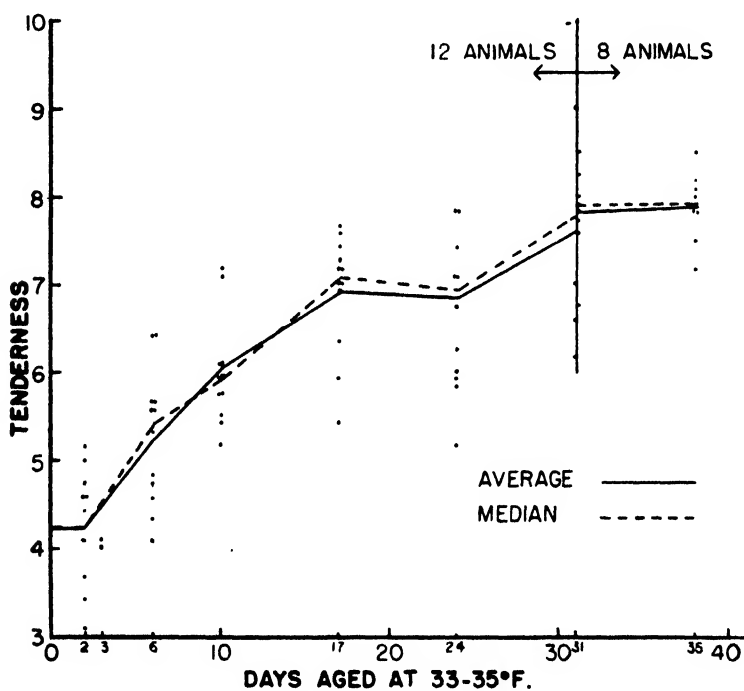
FIG. 5

The foregoing data indicate that there is toughening during certain phases of the aging process, and this too may be explained in terms of C and M. One may imagine that C are strong in some cases so that they maintain their strength from the taster's point of view for some time during the aging period, while at the same time precursors to M, the muscle plasma (P), autolyzes so that on cooking the resulting M are weaker and less firm. Since C are elastic in nature, and if C maintain this elasticity while P changes so that M lose tensile strength, but remain firm or brittle

in character, M would give the taster a mechanical advantage in chewing C by shearing. In much the same way solid fat makes shearing or cutting of raw meat easier at 1.7°C.(35°F.) than the semiliquid fat at 26.7°C. (80°F.). From the mechanical advantage furnished by the firm but brittle or mealy M, C may be effectively masticated by the taster, thus to him the meat is tender. Now if aging proceeds further to the point where C is not yet effectively reduced but where P is autolyzed to form a still less firm M, one can imagine that C has lost the firm or brittle supporting substance. Hence, the mechanical advantage is lost for the taster and to him the meat becomes tough and chewy. It seems significant that some of the tasters participating in this study remarked from time to time quite spontaneously that samples under observation were stringy and chewy, yet the meat had the mealy quality of very tender meat. Going still further with aging, C may lose strength and the meat again increase in tenderness. The work of Steiner (1939) on the physical properties of meat is interesting in relation to this discussion. The foregoing remarks may not be the correct explanations but they are offered as a working hypothesis for subsequent studies.

Taking Group I as a whole it appears that aging at 33-35°F. beyond two and one-half weeks is of little benefit when tenderness alone is considered unless the beef is to be aged longer than four weeks. Of course, in a few cases it was found that tenderness continued to improve beyond 17 days, but to offset this there were some carcasses that definitely became tougher beyond 17 days and did not recover to their 17-day tenderness level until aged 31 or more days. The results, whether considered collectively or individually, cast some doubt on the value of aging meat longer than two and one-half weeks if it is not going to be ripened for more than four weeks.

SUMMARY

Changes in tenderness of 14 beef carcasses during aging at 33-35°F. were determined by estimating after various intervals of time the tenderness of the respective longissimus dorsi muscles by the subjective testing of broiled steaks.

The tenderization curves of 12 carcasses having an initial tenderness of 3.08 to 5.17, inclusive, indicated some variation in the animals used.

The meat from some of the animals became less tender at certain times during the aging period while some of the meat progressively increased in tenderness throughout the aging period. Tenderness of the group increased until 17 days. At 24 days there was no improvement or a slight drop in tenderness, and finally at 31 days there was some improvement beyond the 17-day tenderness level.

The results indicate that unless beef is to be aged at 33-35°F. beyond approximately four weeks it need be aged only two and one-half weeks.

Two animals tested had high initial tenderness of 7.33 and 7.75. Their tenderization curves fell into no general pattern except only small improvement in tenderness was noted. These data are too limited to permit any generalization.

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS

VII. EFFECT OF SUBSTRATE UPON THERMAL RESISTANCE OF SPORES

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Stumbo, Gross, and Vinton (1945b) discussed the influence of meat-curing agents upon the thermal resistance of spores of P. A. 3679 in meat. The experimental procedure was to suspend in sterilized meat spores of P. A. 3679 which had grown in glucose brain broth. Inasmuch as it has been the effort in conducting these studies to correlate experimental laboratory procedure with commercial practice insofar as possible, a critical examination of the experimental procedure in the work of Stumbo, Gross, and Vinton (1945b) is pertinent. The experimental procedure outlined by these investigators does not fully cover all the possibilities that might occur in commercial practice. It is possible that spores which had been previously formed in meat might get into and be distributed throughout another lot of meat. It is also known that putrefactive anaerobes may be present in meat under normal conditions. Jensen (1945) very capably discusses this subject along with the controversial question of the source of such organisms. It is therefore presumed that there is the possibility that putrefactive anaerobic spores might normally be present in meat and that such spores might have formed in a meat substrate. Accordingly it is believed worth while to extend the studies of Stumbo, Gross, and Vinton (1945b) and to determine the thermal resistance of spores of putrefactive anaerobes which have been formed in a meat substrate. Other possibilities are concerned with the condition of the meat, namely, raw, pasteurized, and sterilized, and all of these conditions with and without curing agents in the amounts normally used in comminuted canned meats. If P. A. 3679 is used as the test organism, the technique is relatively simple because the spores of this organism have a greater thermal resistance than the spores of any other mesophilic aerobic or anaerobic sporeformer so far encountered.

From theoretical and practical considerations it would seem unlikely that any extensive contamination or inoculation with anaerobic spores would take place except from conditions where the spores had been formed in meat or meat juices. The formation of spores in glucose brain broth is purely a laboratory technique and may affect the thermal resistance of the spores. The growth of putrefactive anaerobes in meat or meat juices and the formation of spores under unintentional or unavoidable conditions is more likely. It is unlikely that any meats which have been handled under normal conditions of operation both with respect to temperature and time would contain large numbers of spores of putrefactive anaerobes. Spores of putrefactive anaerobes may be present in small numbers and possibly

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those of several species. Aerobic sporeforming organisms are also present and substantial numbers of such organisms may unavoidably be present by the time the comminuted meat product receives the final thermal processing in the hermetically sealed tin container.

Unreported laboratory studies of commercial significance indicate that normal comminuted cured meats in hermetically sealed containers just prior to thermal processing require relatively low levels of thermal processing for complete sterility. This observation has also been extended to include the fact that even though such products may not be sterile in the larger number of cases the spores which survive the thermal processes fail to germinate even after incubation at 23.9 to 29.4°C.(75 to 85°F.) for a period of 12 to 18 months. The data reported by Stumbo, Gross, and Vinton (1945b) is of theoretical importance and contributes to our knowledge of the subject but cannot be directly applied to explain the known facts regarding the bacteriology of commercial meat processing. It would appear that normal content of, contamination with, or artificial inoculation with anaerobic spores does not appear at any level in commercial practice approaching those indicated by the processes necessary in the work of Stumbo, Gross, and Vinton (1945b). It would seem also that either such levels of numbers in the products, inoculation, or contamination do not exist or else the thermal resistance of those organisms which are normally found in regular commercial practice is lower. It is the opinion of the authors that the thermal resistance of the organisms unavoidably occurring in meat under normal conditions of operation is lower than the values indicated by the theoretical work of Stumbo, Gross, and Vinton (1945b).

The senior author (C. Vinton) of this paper observed during the course of earlier investigations that the spores of P. A. 3679 were considerably lower in thermal resistance when grown in raw meat as compared with those grown in glucose brain broth or sterilized meat. This appeared to be a clue that should be followed to some definite conclusion. The present paper is a report based on the investigation arising from this earlier observation.

METHODS AND RESULTS

The methods used were those described by Stumbo, Gross, and Vinton (1945a) with a few modifications and improvements. The meats, organs, and meat products were ground (one-fourth inch plate) and handled using aseptic technique insofar as possible at all times. For those materials which were pasteurized and sterilized, seven ounces were heated in eight-ounce, wide-mouth, screw-cap jars for 135 minutes at 73.9°C.(165°F.) and 90 minutes at 121.1°C.(250°F.), respectively. Where indicated, sterilized curing agents were added in the proportion of salt, 3½ lb.; sugar, 1 lb.; sodium nitrate, 2¾ oz.; and sodium nitrite, ¼ oz. per hundredweight of meat. Unless otherwise indicated the inoculation was one milliliter per 100 grams of a spore suspension of S_2 (P. A. 3679), as described by Stumbo, Gross, and Vinton (1945a); after mixing thoroughly the jars were incubated at 37°C.(98.6°F.) for eight days. If not used immediately after incubation the jars were placed under refrigeration until used. Spore counts were made after pasteurization using the decimal serial dilution

technique. Three tubes were used at each processing level. A Precision (No. 6546) oil bath was used and the temperature was maintained within 0.17°C.(0.3°F.) by means of a sensitive mercury-type thermoregulator. All tubes were processed at 114.5°C.(238.1°F.) unless otherwise indicated. The processed tubes were cooled by transferring immediately to water at 21.1°C.(70°F.). The processed and cooled tubes were subcultured into two-ounce, screw-cap bottles containing 30 ml. of glucose brain broth and glass beads. A short time in a mechanical shaker was sufficient to disin-

TABLE 1

Thermal Resistance of Spores Formed in Meat

Media in which growth and spore formation occurred	Spores of P.A. 3679 per gram	F value (z = 16) Survived	Destroyed	Media in which growth and spore formation occurred	Spores of P.A. 3679 per gram	F value (z = 16) Survived	Destroyed
Raw meat— uncured				Raw meat— cured			
Blade meat........	10,000	....	1	Blade meat........	10,000	1	2
Blade meat........	10,000	2	3	Blade meat........	10,000	....	1
Blade meat........	10,000	1	2	Blade meat........	10,000	1	1.5
Blade meat........	10,000	2	3	Pork (20% fat)...	10,000	2	3
Blade meat (pH 6.9)........	10,000[1]	2	3	Pork (20% fat)...	10,000	4	5
Blade meat........	10,000	4	5	Pork (40% fat)...	2 x 10^6	3	4
Blade meat........	10,000	3	4	Pork (20% fat)...	3 x 10^6	3	4
Ham....................	10,000	3	4	Average...........	....	2	2.9
Ham....................	1 x 10^5	2	3	Pasteurized meat— uncured			
Ham....................	6 x 10^6	4	5	Blade meat........	10,000	6	8
Blade meat........	1 x 10^7	3	4	Blade meat........	10,000	8	10
Pork butts........	2 x 10^7	4	5	Ham....................	10,000	8	10
Blade meat........	5 x 10^7 [2]	1	2	Ham....................	5 x 10^6	10	—(12)[3]
Blade meat........	1 x 10^8	2	3	Blade meat........	7 x 10^6	14	—(16)[3]
Blade meat........	1 x 10^8	1	2	Ham....................	8 x 10^6	8	10
Blade meat........	1 x 10^8	2	3	Ham....................	4 x 10^7	8	10
Beef trimmings...	3 x 10^8	3	4	Average...........	....	8.9	10.9
Ham....................	4 x 10^8	3	4	Sterilized meat— uncured			
Ham....................	7 x 10^8	4	5	Ham....................	10,000	10	12
Blade meat........	7 x 10^8	3	4	Blade meat........	10,000	6	8
				Blade meat........	10,000	12	—(14)[3]
Average...........	....	2.5	3.5	Ham....................	2 x 10^7	16	—(18)[3]
				Blade meat........	6 x 10^7	12	14
				Ham....................	4 x 10^8	5	6
				Average...........	....	10.1	12.0

[1] Inoculated with spores which had resisted process F 8.   [2] Plus 3 x 10^5 *Staphylococcus* No. 184.
[3] End point not reached; value assumed.

tegrate the solid contents of the bottles. The bottles were then incubated at 37°C. for eight days and growth was determined by the following criteria: digestion of the meat and brain, darkening of the brain, gas formation, odor, and microscopic examination. The results for thermal resistance were recorded as F values (z equals 16) for the last processing level showing any positive tubes and the first processing level showing all negative tubes.

Thermal Resistance of Spores Formed in Meat: The thermal resistance of the spores formed in meat varied widely (Table 1). For 20 samples of

raw meat without cure the average F values for survival and destruction were 2.5 and 3.5, while for seven samples with cure 2.0 and 2.9 were found. For seven samples of pasteurized meat without cure the average F values for survival and destruction were 8.9 and 10.9. For six samples of sterilized meat without cure the average F values were 10.1 and 12.0. For sterilized meat with cure values were found to be in the same range, indicating that cure apparently has little or no effect on thermal resistance. Typical data for sterilized meat with cure are reported in the section on the effect of substrate on the slope of the thermal death time curve.

TABLE 3

Thermal Resistance of Spores Formed in Commercial-Type Meat Products

Product in which growth and spore formation occurred	Spores P. A. 3679 per gram	pH	Thermal resistance F (z = 16)	
			Survived	Destroyed
Liver pudding				
Raw..	4×10^3	4.1	....	<1
Pasteurized.......................................	5×10^5	4.6	6	7
Sterilized..	2×10^4	5.8	6	7
Liver sausage				
Raw..	3×10^6	6.0	3	4
Pasteurized.......................................	6×10^7	6.0	11	12
Sterilized..	2×10^6	5.8	6	7
Luncheon meat (20% fat)				
Raw..	5×10^5	5.5	4	5
Pasteurized.......................................	6×10^4	5.6	9	10
Sterilized..	8×10^4	6.4	9	10
Pork sausage (40% fat)				
Raw..	6×10^6	7.1	4	5
Pasteurized.......................................	9×10^5	6.5	7	8
Sterilized..	1×10^7	6.5	9	10
Pork sausage (50% fat)				
Raw..	2×10^6	5.7	4	5
Pasteurized.......................................	9×10^5	6.5	7	8
Sterilized..	6×10^7	6.4	7	8
Average				
Raw[1]...	....	....	3.8	4.8
Pasteurized.......................................	....	....	8	9
Sterilized..	....	....	7.4	8.4

[1] Omitting liver pudding.

Thermal Resistance of Spores Formed in Organ Tissue: The thermal resistance of spores formed in organ tissue was found to vary widely (Table 2). For six different organ tissues the average F values for survival and destruction were as follows: raw, 3.3 and 4.3; pasteurized, 11.3 and 12.3; and sterilized, 12 and 13. When curing agents were present the values were as follows: raw, 3.7 and 4.7; pasteurized, 10.3 and 11.3; and sterilized, 8.5 and 9.5.

Thermal Resistance of Spores Formed in Meat Products: Representative types of meat products were selected and the required amount of the meat product was taken from a load in the plant. The data (Table 3) show that the thermal resistance expressed as average F values for survival

TABLE 2

Thermal Resistance of Spores Formed in Organ Tissue

Media in which growth and spore formation occurred	Cure added	Raw						Pasteurized						Sterilized					
		pH		Spores P.A. 3679	F value ($z=16$)		pH		Spores P.A. 3679	F value ($z=16$)		pH		Spores P.A. 3679	F value ($z=16$)	
		Initial	8 days		Surv.	Dest.	Initial	8 days		Surv.	Dest.	Initial	8 days		Surv.	Dest.		
Brains, sheep	No	6.7	7.7	3×10^8	4	5	6.9	8.0	1×10^8	13	14	7.05	8.05	5×10^7	15	16
Brains, sheep	Yes	6.3	7.7	2×10^7	6	7	6.7	5.8	6×10^7	8	9	7.0	5.0	5×10^6	11	12
Hearts, beef	No	6.2	7.2	7×10^7	2	3	6.5	7.1	2×10^8	11	12	6.5	7.1	2×10^7	12	13
Hearts, beef	Yes	5.6	6.3	3×10^5	4	5	6.3	6.3	2×10^6	11	12	6.2	6.3	2×10^5	8	9
Liver, beef	No	6.4	7.6	9×10^5	3	4	6.5	7.6	9×10^5	8	9	6.3	7.2	7×10^5	8	9
Liver, beef	Yes	6.2	5.5	2×10^5	2	3	6.4	7.1	4×10^5	8	9	6.2	6.1	3×10^5	6	7
Lungs, beef	No	6.6	7.2	2×10^8	5	6	6.9	8.2	3×10^8	14	15	6.8	7.3	1×10^7	10	11
Lungs, beef	Yes	6.4	8.3	8×10^5	4	5	6.6	8.2	9×10^5	12	13	6.6	7.5	5×10^5	7	8
Stomachs, pork	No	6.6	7.3	3×10^5	3	4	6.9	7.5	1×10^5	15	16	7.0	8.0	3×10^5	16	....(18)[1]
Stomachs, pork	Yes	6.5	7.5	3×10^4	2	3	6.9	6.2	6×10^5	14	15	6.9	5.1	3×10^5	10	11
Tripe, beef	No	7.6	7.7	3×10^8	3	4	7.8	7.8	7×10^8	7	8	7.8	7.5	2×10^5	11	12
Tripe, beef	Yes	7.5	6.9	2×10^7	4	5	7.5	6.4	6×10^7	8	9	7.2	6.0	5×10^6	9	10
Average	No	....	....	....	3.3	4.3	....	....	....	11.3	12.3	....	....	....	12	13
Average	Yes	....	....	....	3.7	4.7	....	....	....	10.3	11.3	....	....	....	8.5	9.5

[1] End point not reached; value assumed.

TABLE 6

Effect of Substrate on Slope of Thermal Death Time Curve

Medium in which spores formed	Organism	Spores per gram	pH	Minutes[1] for destruction at temperature (°F.)						F for destruction	z in °F.
				208.9	217.4	225.6	232.2	238.1	246.4		
Raw meat with cure[2]	P. A. 3679	7×10^5	5.3	2114	550	181	64.8	27.8	8.4	4.2	15.2
Sterilized meat with cure	P. A. 3679	2×10^6	5.1	3882	880	326	184.0	50.2	13.0	7.7	15.2
Raw meat with cure	Cl. sporogenes (Spray)	8×10^6	6.0	1602	398	119	39.0	14.4	2.9	2.3	14.8
Sterilized meat with cure	Cl. sporogenes (Spray)	2×10^7	6.3	2107	556	185	56.0	22.1	8.5	4.1	15.1

[1] Correction for heating and cooling lag subtracted. [2] Cure: salt, 3½ lb.; sugar, 1 lb.; sodium nitrate, 2% oz.; and sodium nitrite, ⅞ oz. per hundredweight of meat.

and destruction were raw, 3.8 and 4.8; pasteurized, 8 and 9; and sterilized, 7.4 and 8.4.

Effect of Medium in Which Spores Are Formed Upon Their Thermal Resistance: In this series of experiments spores of P. A. 3679 were allowed to form in raw meat with and without cure and in raw organ tissue. Thermal resistance tests were then made. Transfers of one gram of each of these materials were made to glucose brain broth and the cultures were incubated at 37°C.(98.6°F.) for eight days. Sufficient spores were taken from the glucose brain broth suspensions and mixed in raw meat to give a

TABLE 4

Effect of Medium Upon Thermal Resistance of Spores Formed

First medium		Second medium	Thermal resistance; F value ($z = 16$)			
Grown and heated in	Spores	Grown in glucose brain broth; heated in	First medium		Second medium	
			Survived	Destroyed	Survived	Destroyed
Blade meat	1×10^4		1	2	6	8
Blade meat	1×10^4		1	2	8	10
Ham	1×10^5		2	3	6	8
Blade meat (pH 5.9)	3×10^5		1	2	6	8
Blade meat	2×10^7	.	1	2	8	10
Blade meat	3×10^7		1	2	8	10
Blade meat	3×10^7	Raw meat—	2	3	6	8
Ham	3×10^8		3	4	6	8
Beef trimmings (pH 6.8)	3×10^8	10,000	4	5	6	7
Average	....		1.8	2.8	6.7	8.7
Blade meat, cured	1×10^4	spores	1	2	8	10
Blade meat, cured	8×10^5	per gram	1	2	8	10
Blade meat, cured	2×10^7		1	2	8	10
Blade meat, cured	3×10^7	on all	1	2	12	14
Average	....		1	2	9	11
Pork liver	1×10^5		3	4	5	6
Pork hearts	3×10^7		4	5	7	8
Beef spleen	2×10^8		4	5	5	6
Beef lungs	3×10^8		5	6	6	7
Average	....		4	5	5.8	6.8
Over-all average	....		2.1	3.1	7.0	8.7

concentration of 10,000 per gram. The thermal resistance of the spores which had formed in glucose brain broth was then determined. The data from this series of experiments (Table 4) show that the average F values for survival and destruction for spores formed in raw meat were 1.8 and 2.8 and when cure was added to the raw meat the values were 1 and 2. The thermal resistance of the spores formed in glucose brain broth after transfer from raw meat gave an average F value of 6.7 survived and 8.7 destroyed, while those grown in glucose brain broth after transfer from raw meat with cure gave an average F value of nine survived and 11 destroyed. The spores formed in glucose brain broth after transfer from organ tissue gave an average F value of 5.8 survived and 6.8 destroyed which occur in the same range as those found for spores from glucose

brain broth after transfer from raw meat and raw meat with cure although they are somewhat lower. The difference in thermal resistance between the spores formed in glucose brain broth and those formed in organ tissue is not very great and not nearly so great as that between spores formed in glucose brain broth, in raw meat, and in raw meat with cure. The values found for the thermal resistance of the spores formed in raw organ tissues are the same order as those found previously and recorded (Table 2). However, the resistance of the spores grown in glucose brain broth after transfer from growing in raw organ tissue seems to be somewhat lower .than is normally found for spores growing in glucose brain broth.

A control determination was included in this series of experiments. The control consisted in determining the thermal resistance of the spores formed in glucose brain broth after several consecutive transfers. In three such determinations the thermal resistance was found to be an F value of eight survived and 10 destroyed in all three experiments.

Effect of Transfers From Medium to Medium Upon Thermal Resistance: The standard suspension of S_2 (P. A. 3679) was used and one ml. per 100 grams was added as inoculum to aseptically prepared raw blade meat. The inoculated meat was incubated eight days at 37°C. Thermal resistance determinations were then made. Both glucose brain broth and aseptically handled raw meat were inoculated from this meat using one gram to 30 ml. and one gram to 100 grams, respectively. After incubation eight days' thermal resistance determinations were made. One gram of the meat was used as inoculum for 100 grams of aseptically handled raw blade meat and 30 ml. of glucose brain broth and incubated eight days again. Thermal resistance determinations were made and one gram was used as inoculum for 30 ml. glucose brain broth. After eight days' incubation thermal resistance determinations were made. The thermal resistance determinations on all spores formed in glucose brain broth were made by suspending the indicated number of spores in aseptically prepared raw blade meat. The last transfer of raw meat to brain broth was lost through accidental autoclaving of the glucose brain suspension before thermal resistance determinations had been made. The experiment was repeated. The data for the two experiments are shown (Table 5) and times of survival and destruction are given as F values assuming a z value of 16°F. The average F values for spores formed in meat were 2.1 survived and 3.0 destroyed, while the average F values for spores formed in glucose brain broth were 7.6 survived and 9.2 destroyed.

Effect of Substrate on Slope of Thermal Death Time Curve: The slope of the thermal death time curve was determined for spores of S_2 (P. A. 3679) formed in raw meat plus cure and in sterilized meat plus cure. Nine tubes were used at each processing level for six temperatures and the data are shown (Table 6). The F values were 4.2 and 7.7 for raw and sterilized meat, respectively, while the z value was constant at 15.2°F. for both.

The slope of the thermal death time curve of spores of *Clostridium sporogenes* (Spray) was also determined. The source of the culture was described by Gross, Vinton, and Stumbo (1946). The average times for four different determinations are shown (Table 6), and the F values

TABLE 5

Effect of Repeated Transfers Upon Thermal Resistance

Blade meat (1×10^7)
Survived 1
Destroyed 2

Brain (1×10^6)
Survived 8
Destroyed 10

Blade meat (1×10^6)
Survived 4
Destroyed 6

Brain (1×10^6)
Survived 8
Destroyed 10

Blade meat (2×10^6)
Survived 1.5
Destroyed 2.0

Brain
lost accidentally

Blade meat (2×10^7)
Survived 4
Destroyed 5

Brain (2×10^7) (pH 7.0)
Survived 7
Destroyed 8

Blade meat (4×10^7) (pH 5.9)
Survived 1.5
Destroyed 2.0

Brain (2×10^7) (pH 5.9)
Survived 8
Destroyed 10

Blade meat (4×10^6) (pH 5.7)
Survived 0.5
Destroyed 1.0

Brain (1×10^6) (pH 6.4)
Survived 7
Destroyed 8

obtained were 2.3 and 4.1, respectively, for raw and sterilized meat with cure. The z values were 14.8°F. and 15.1°F. for raw and sterilized meat with cure, respectively. These values can be considered identical within the limits of the experimental error.

DISCUSSION

It is apparently a well-known fact often observed and commented upon that the resistance of spores is variable and dependent upon many factors among which are the substrate in which the spores were formed. Esty and Meyer (1922) state that spores which develop in certain mediums are always of very low resistance and that supplementary tests have further-more shown that striking variations are obtained when the spore suspensions are prepared and heated in the culture fluid in which they have been produced. Buchanan and Fulmer (1930) state that composition and char-acteristics of the medium are important in determining the thermal death rate: "The medium in which sporulating bacteria have been grown is of importance in determining the thermal death time. This is well shown by the results of Esty and Meyer (1922) who worked with spores of *Clos-tridium botulinum* grown in various media. The thermal death times were found to vary as much as 100 per cent."

Rahn (1945) states that the medium in which bacteria are suspended can influence the death rate. The pH value of the medium is of great importance but often differences in pH alone cannot explain differences in two media. Cameron, Esty, and Williams (1936) make an interesting com-ment regarding possible loss of original high thermal resistance of spores through laboratory manipulations.

The comments of Tanner (1944) are also of interest. Tanner lists sep-arately a number of factors known to affect the thermal death time values among which is the reaction of the menstruum in which spores are heated and resistance was the greatest close to the neutral point: "According to Dickson *et al.* (96) spores propagated in a neutral medium showed greater heat resistance than those from acid or alkaline media." Tanner also reports: "Esty and Meyer (1922) observed that the heat resistance of spores of *Clostridium botulinum* varied considerably even from the same strain and that while some factors are known others were unknown. The heat resistance of spores of *Clostridium botulinum* in 17 varieties of canned food juices showed a variation of from less than ten minutes to 230 minutes at 100°C. (212°F.)."

In this report evidence of the variation of thermal resistance of spores owing to substrate has been advanced. No mechanism is suggested to ex-plain this variation. Unpublished work from this laboratory shows that this effect can be demonstrated for other sporeforming organisms both aerobic and anaerobic.

It is suggested that the variation of thermal resistance of spores owing to substrate and the inhibition of germination of spores owing largely to salt may be used to correlate theoretical bacteriological data and the commercial results obtained in meat canning. Under ordinary conditions of commercial practice viable spores may be present in the finished product

in rather small numbers and germination is inhibited by the presence of salt and other meat-curing agents. Under unusual conditions either the number or the thermal resistance of the normal number or both permit a larger number of spores to survive the standard thermal processing. Thus the number of spores may be greater in the finished product and the limit of inhibitory effect on germination by the salt and curing agents may be reached. Stumbo, Gross, and Vinton (1945c) show that the inhibitory effect is directly related to numbers of spores present. An explanation such as proposed would serve to explain the usual low percentage of spoilage in a given lot because while the average number of spores was approaching that beyond which salt and curing agents would no longer inhibit germination, the possibility is good that greater numbers and lower salt content in small volumes of the product might exist. Under such conditions a few cans per thousand might show spoilage and such spoilage might not be apparent for several months under ordinary storage conditions.

It is not the intention of the writers to minimize the number of factors yet unknown or the need for continued research on this problem. Rather it was the intention to point out the possibility that the two factors of variation in thermal resistance owing to substrate and inhibition of germination owing to curing agents might be the connecting links to rationalize bacteriological data with the results obtained in commercial practice. Additional work should be done especially to extend the observations and more especially to include work on the public-health aspects. The commercial results and theoretical bacteriological data do not agree on this subject either. Another angle which needs further study is the determination of the slopes of the thermal death time curves for the important organisms concerned in meat processing both from spoilage and public-health aspects. In the experience of the writers no values of 18°F. for the slope of the thermal death time curve for anaerobes in meat have yet been found although this value is normally assumed when no better information is at hand. Such an assumption when the value may actually be 16°F. causes an error of 100 per cent in the calculation of the lethal value of a process at a temperature of 100°C. (212°F.). Of course there is no difference in process values at 121.1°C.(250°F.) and the error becomes less as temperatures approach 250°F.

SUMMARY

The effect of substrate upon the thermal resistance of spores formed in it has been demonstrated for P. A. 3679 in meat, organs, mixtures of meats, and for mixtures of meats and organs. The thermal resistance of the spores is less when grown in raw meat or organs and increases when grown in either pasteurized or sterilized meats and organs. The average increase amounts to approximately three times.

The effect was shown to be related to the substrate itself through repeated raising and lowering of resistance by transfer from media to media.

The substrate is shown to have no effect on the slope of the thermal death time curve for P. A. 3679 or for *Clostridium sporogenes* (Spray.)

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS

VIII. THERMAL RESISTANCE OF SPORES NORMALLY PRESENT IN MEAT

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Previous papers in this series were concerned with theoretical studies attempting to determine the influence of various factors upon thermal processing using spores of P. A. 3679 as the test organism. Every effort was made to correlate the laboratory scale studies with commercial practice so that the results obtained could more readily be evaluated with respect to possible practical applications. Even so there appeared to be some unavoidable differences between laboratory technique and commercial practice. One important difference was the fact that a known number of spores of P. A. 3679 were used as inoculum whereas under commercial conditions the numbers and species are unknown and probably variable.

An effort was made to find some practical method to gain information regarding the bacteriological condition of the meat products just prior to thermal processing. The point of greatest concern was thought to be the level of thermal resistance of the spores present in the meat product. Further, by a process of fractionation by thermal resistance the bacteriological condition of the product could be evaluated with respect to spores of organisms having a reasonably high thermal resistance. Such a method was developed and is described in detail. Typical results obtained from a weekly survey of three plants over a period of 18 months are also presented.

EXPERIMENTAL PROCEDURE

The general technique used was described by Stumbo, Gross, and Vinton (1945a); however, it is thought worth while to review it in detail because some slight changes have been made.

The samples were secured by taking hermetically sealed cans from a production line just prior to thermal processing. The samples were handled immediately or, if from another plant, were immediately placed in dry ice and packed for shipment. Sufficient dry ice was used so that a reserve supply still remained upon arrival at the laboratory. The frozen samples were defrosted by holding in running tap water at approximately 21.1°C.(70°F.) for sufficient time; for example, two hours were sufficient for rectangular cans 402 x 310 x 1208 containing six pounds of meat.

The cans were opened and the top half of the meat removed with sterile equipment in an aseptic manner. The center portion was removed and placed in the chamber of a sterile Alemite gun fitted with a needle suitable for stuffing 10 x 75 mm. chemical test tubes (Kimble No. 45050) with approximately one gram of meat. The tubes were then sealed in a blast lamp forming a loop at the top so that a number of tubes could be joined

together with a safety pin for simplicity of handling and identification during the processing and cooling operations.

The tubes were then processed in an oil bath (Precision No. 6546) controlled to within 0.17°C.(0.3°F.) of any desired temperature for the necessary time to secure the desired process values corrected for the heating and cooling lag. For example, 40 tubes were processed at 101.2°C. (214.2°F.) using 10 tubes at each processing level of 0.05, 0.2, 0.6, and 1.0 F_o values corresponding to 421, 1,341, 3,795, and 6,249 seconds actual heating time, respectively. The total time for a process value of F_o 1 was obtained by determining the factor F_1 at 214.2°F. by reference to the tables of Ball (1928) assuming a value for z of 18°F. which is a customary commercial practice in process calculations. The value of F_1 was found to be 102.24 and therefore 102.24 minutes would be required to which must be added the heating and cooling lag correction of 1.91 minutes making a total time of 104.15 minutes or 6,249 seconds. The cooling operation consisted of immediately. transferring the tubes to a water bath of 21.1°C. (70°F.) when the process in the oil bath was complete.

After processing and cooling six tubes were subcultured into 30 ml. of glucose brain broth contained in two-ounce, screw-cap bottles also containing glass beads. The bottles were shaken to disintegrate the particles of meat and were then incubated for a total time of 14 days at 37°C. (98.6°F.). Observations were made at intervals to determine whether there was any evidence of growth. Growth was determined by the following criteria: digestion of the meat and brain, darkening of the brain, gas formation, odor, and microscopic examination. Four unopened tubes were held at room temperature of 23.9 to 29.4°C.(75 to 85°F.) for observation over a period of 18 months. Visual inspection was used as an indicator of bacterial activity in the meat. The confirmatory test used was subculture into glucose brain broth for tubes showing visual evidence of activity and for those in the doubtful classification.

RESULTS

The data are summarized (Table 1) for the subculture of five to six tubes and for the incubation of three to four tubes from each processing level for each sample. The incubation period was at a room temperature of 75 to 85°F. for nine to 18 months and visual inspection showed no definite evidence of spoilage in any of the tubes at any processing level for any period of incubation, although at the end of the 18 months' incubation three tubes were suspected inasmuch as they had either lost their original color or were discolored. These tubes were subcultured with negative results. The records indicate that duplicates of these tubes were also negative when subcultured immediately after processing. Therefore after nine to 18 months of incubation there was no confirmed visual evidence of spoilage. A few tubes showing suspicious loss of color or showing discoloration are among the group which have not yet reached the 18 months' incubation period at which time they will be opened and subcultured. A random sampling of the tubes which have passed through the 18-month incubation period indicated that the spores were still viable

in the tubes which were duplicates of those showing viable spores when subcultured immediately after processing. The reason for not subculturing all of these tubes is that it is planned to hold the larger part of them for an indefinite period in the future to serve as a check on whether or not delayed germination as long as two or three years might occur and also to check whether or not the spores will remain viable over similar periods.

TABLE 1

Thermal Resistance of Spores Normally Present in Canned Meat

Product	Plant	Number of samples	Number of samples sterile at F_0			Subculture after process				Visual inspection; incubated at 75 to 85°F. from 9 to 18 months	
						Tubes processed each level	Tubes positive F_0			Number of tubes	Number positive at F_0
			0.2	0.6	1.0		0.2	0.6	1.0		0.2 0 6 1.0
Pork luncheon meat[1] in 12-oz., 2½-, and 6-lb. cans	A	45	12	22	37	253	130	64	17	169	
	B	28	3	14	19	154	103	55	35	105	
	C	33	12	15	24	181	73	52	37	122	None on any
Chopped ham[2] in 6- and 8-lb. cans	A	9	0	5	8	47	23	11	6	32	
	B	8	1	0	3	45	23	21	14	30	
Bulk pork sausage[3] in 24- and 34-oz. cans	A	9	2	1	4	46	26	31	9	32	
	B	12	1	5	7	62	37	17	9	41	

[1] Composition: picnics, butts, lean trimmings (20% fat), and blade meat in order of diminishing percentages. Salt content, 2.9-3.4%; fat content, 20-22%. [2] Composition: heavy hams. Salt content, 2.8-3.8%; fat content, 18-20%. [3] Composition: lean (20% fat) and regular (50% fat) pork trimmings. Salt content, 2.2-2.6%; fat content, 25-40%.

The data show that the percentage of tubes showing viable spores decreases with the increase of thermal process value as might be expected. There appeared to be significant differences in the results on pork luncheon meat since a lower percentage of positive tubes was observed throughout for Plant A. It is also especially significant that the percentage of positive tubes for Plant A at F_0 1 is approximately one-third the values for Plants B and C. The data for chopped ham also show a difference favorable to Plant A. It is interesting to note that apparently the conditions of handling were reversed in favor of Plant B for bulk pork sausage.

On a part of the samples a processing level of F_0 0.05 was also used; these samples are not included in the data in Table 1. Considering all products 326 tubes were processed at this level and 296 were positive when subcultured. There were also 185 tubes held for the incubation period which at the end of 18 months did not show any visible evidence of spoilage although subculture of a few tubes indicated that viable spores were still present.

DISCUSSION

It is interesting to note that for the pork luncheon meat containing 2.9 to 3.4 per cent salt processed tubes were positive upon subculture as follows: 7 to 23 per cent at F_0 1, 25 to 35 per cent at F_0 0.6, and 52 to 65 per cent at F_0 0.2. Yet, although the duplicate tubes have been incu-

bated at 75 to 85°F. for nine to 18 months, there is no visual evidence of spoilage. The previous experience of Stumbo, Gross, and Vinton (1945c) indicated that visual observation correlated directly with germination of spores and growth as did also organoleptic observations on the tubes after opening.

Since there was no visual evidence of growth (spoilage) in any of the tubes at processing levels of F_0 0.05, 0.2, 0.6, and 1.0 and yet there were viable spores present at the beginning and end of the incubation period, the conclusion seems warranted that there has been inhibition of germination. Observations of Stumbo, Gross, and Vinton (1945b) would seem to indicate that the salt concentration might be the most important single factor inhibiting germination of spores in canned meat products. It is also probable that the numbers of viable spores are low, and so relatively low amounts of salt such as were used in bulk pork sausage would still inhibit germination and growth. The data for chopped ham is kept separate because it seemed that possibly hams might show a higher number of heat-resistant, sporeforming organisms because the ham is the last part of the carcass to be chilled completely after slaughter. The small amount of data available do not permit any final conclusions to be drawn but there appears to be an indication that the chopped ham was in about the same bacteriological condition as the pork luncheon meat.

The data for bulk pork sausage are of interest because the salt content was somewhat lower than on the other two products and yet there was no visual evidence of growth on the tubes held at room temperature for over nine months. It would appear that the per cent of tubes showing viable spores at the lower processing levels is higher than for pork luncheon meat.

It is the opinion of the authors that this work should be regarded as preliminary and possibly indicating a promising angle of attack upon the problems relating to the thermal processing of canned meat. However, no definite or final conclusions should be drawn because this work should be checked and considerably extended before sufficient data are available to justify final conclusions. It is also the opinion of the authors that an attempt should not be made to interpret this work as indicating that low thermal processes may be commercially safe or that inhibition by salt can be depended upon on a commercial scale in preventing spoilage. A vast amount of work yet remains to be done and to be checked by others on an industry-wide basis before such conclusions are warranted.

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THERMAL DEATH TIME OF A STRAIN OF *STAPHYLOCOCCUS* IN MEAT

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The problem of the destruction of bacteria by heat has interested many investigators. Major attention has been directed toward determining such data for spore-bearing organisms because of their relationship to the spoilage of canned foods as well as because of theoretical considerations. The thermal death time of nonsporeforming organisms is also of commercial significance in connection with pasteurization processes. Beamer and Tanner (1939) present an excellent review of the historical background of thermal resistance studies as well as an unusually complete bibliography.

Stritar (1941) presented data on the thermal death time of several strains of food-poisoning staphylococci in curing solutions and in meat with and without the addition of curing agents. No other references to the determination of thermal resistance of staphylococci in meat were found. Therefore it was thought that further work might be of value both to confirm the work of Stritar and to extend it.

Gross and Schaub (1945) suggested a new method for evaluating the lethality of pasteurization processes for meat products. Their arbitrary value F' will be used to express the lethality of the pasteurization processes necessary to destroy an artificially inoculated, heat-resistant, food-poisoning strain of staphylococci in meat. In order to use the F' value the thermal death time curve must be known, determined, or assumed. The data of Stritar (1941) can be calculated for this purpose and Ball (1943) has already calculated the data of Beamer and Tanner (1939) in this manner. Inasmuch as some assumptions must be made regarding the rate of heat penetration in connection with the work of Stritar, and Beamer and Tanner did not use a meat substrate, it was decided to attempt to determine the thermal death time for the heat-resistant strain of food-poisoning *Staphylococcus* No. 184 in meat. It was decided to use the method described by Stumbo, Gross, and Vinton (1945a). This method gives absolute thermal death times by using the end-point technique.

In the determination of the thermal resistance of an organism many factors may affect the results obtained. Important among these is the medium in which the organisms were grown and in which they are suspended for the thermal processing. In order that laboratory data may have significance when the attempt is made to translate it into terms of commercial practice it is important that the experimental procedure be as closely related to commercial practice as is possible. The major problems in experimental technique arose because of the effort to conduct the laboratory experiments under conditions comparing closely with commercial practice.

After a meat product is artificially inoculated with staphylococci they they may or may not grow under normal conditions during the period prior to thermal processing. If the organisms grow in the meat substrate their thermal resistance after growth may be altered, and therefore it is important to include this possibility in any study. Whether or not the artificially inoculated organisms grow under normal conditions in the particular meat product under investigation is a problem in itself. In this investigation the point of major interest is determining the thermal resistance of staphylococci in meat. From a theoretical standpoint it may be assumed that there is the possibility of growth under some unusual conditions. The problem therefore appears to fall into three major subdivisions as follows: first, the thermal death time of staphylococci artificially inoculated into meat; second, the thermal death time of staphylococci artificially inoculated into and growing in meat; and third, a limited study of the possibilities of growth of artificially inoculated staphylococci in meat products under normal conditions of handling under refrigeration.

Tanner (1944) states that evidence indicates that organisms in old cultures appear to be more heat resistant, so both 48-hour and 14-day cultures were used. In the determination of the thermal death time of staphylococci grown in meat for 48 hours and for 14 days there appeared to be a number of possible combinations which might exist in the product at the time of inoculation. These conditions were presumed to be with and without curing agents in raw, pasteurized, and sterilized meat.

For demonstration of the identity of isolated organims thought to be *Staphylococcus* No. 184 no fully reliable and reproducible test was available except possibly the use of susceptible human volunteers. The Dolman kitten test was not found to be fully reliable even on known materials. During a part of the investigation the diagnostic media proposed by Chapman (1945) was used. This media is a phenol red mannitol agar with 7.5 per cent salt content. It was found to be excellent for differentiating coccal forms by suppressing the growth of the other forms. *Staphylococcus* No. 184, as well as a number of other coccal forms common to packing-house flora, grew luxuriantly on this media. From the scant evidence available there was no conclusion warranted that this media is suitable as a diagnostic media for pathogenic staphylococci.

METHOD AND RESULTS

Organism, Inoculum, and Technique: The test organism No. 184 was obtained from Dr. J. E. Stritar of the Research Laboratories of the American Meat Institute, Chicago, Illinois. Dr. Stritar stated that it was the most heat resistant of 16 strains obtained from Dr. G. M. Dack of the Department of Bacteriology, University of Chicago. These strains were known to produce enterotoxin and cause *Staphylococcus*-type food poisoning in monkeys and kittens as well as in human volunteers. The organism grows well in plain broth and in meat alone or with curing agents. Dack (1943) states that strains of *Staphylococcus* grow well in cured meats and in salt meat containing five to 10 per cent salt which inhibits the growth of sporeforming and nonsporeforming rods. This was confirmed

and the effect was later utilized in helping to isolate staphylococci from mixed cultures.

The broth cultures were produced by inoculating proteose-peptone salt broth (five gm. proteose-peptone, five gm. dextrose, three gm. beef extract, 65 gm. salt, made to one liter, adjusted to pH 7) with *Staphylococcus* No. 184 and incubating at 37°C.(98.6°F.) for 48 hours and for 14 days. This broth will be referred to hereafter as salt broth.

The general method was fully described by Stumbo, Gross, and Vinton (1945a). Lower processing temperatures were used but oil was retained as the heating medium. The initial temperature of the tubes was 6.1°C. 43°F.) and the cooling medium was water at 21.1°C.(70°F.). Determination of the rate of heat penetration in meat showed that it was normal and proceeded by conduction. The correction for heating and cooling lag was determined, using the methods suggested by Sognefest and Benjamin (1944). The correction was three minutes within the experimental error for the 10 x 75 mm. chemical test tubes containing meat over the temperature range of 57.8 to 68.9°C.(136 to 156°F.). The method of determination of the end point of survival had to be changed in some cases and is presented in detail where changes were necessary.

In order to calculate the time of heating at any chosen temperature to correspond to a given time at 65.6°C.(150°F.) the factor F_1' was determined assuming a value of 9 for z. The equation is F_1' equals $\log^{-1}$ (150-T)/z where T is the processing temperature being used and z is the slope of the thermal death time curve known, assumed, or determined; values of F_1' for various temperatures and values of z are shown (Table 1).

The pH of the raw meat, with and without cure, was in the range 5.6 to 5.8 and of the pasteurized and sterilized meat, with and without cure, in the range 5.9 to 6.1. Different pH values were sometimes found especially when the staphylococci had grown in the meat. Typical values are shown (Table 4) for both pH and numbers of organisms after 48-hour and 14-day incubation.

The terms cured or curing agents added refer to the addition of sterilized curing agents in the proportion of 3½ lb. salt, 1 lb. sugar, 2¾ oz. sodium nitrate, and ¼ oz. sodium nitrite per hundredweight of meat.

Meats referred to as pasteurized and sterilized were treated by heating seven ounces in an eight-oz., wide-mouth, screw-cap bottle for 135 minutes at 73.9°C.(165°F.) and 90 minutes at 121.1°C.(250°F.), respectively.

Thermal Death Time of Staphylococci Suspended in Meat: The lean meat was separated from a fresh ham using aseptic technique and dividing the meat into two equal portions. Sufficient 48-hour salt-broth culture was added to each to give a concentration of 10,000 organisms per gram, and to one portion was also added sterilized curing agents. These portions were then ground, mixed, and reground through a sterile grinder with aseptic technique in order to distribute the organisms and curing agents evenly throughout the meat. The foregoing was repeated using 14-day salt-broth cultures. Similar treatment was given to fresh lean ham which had been sterilized.

Sterilized lean ham prepared as described before was used with sterilized curing agents added and the amount of 48-hour salt-broth culture of

TABLE 1

F_i' Values for Various Temperatures and Values of z

[Calculated from formula: $F_i' = \log^{-1}(150\text{-}T)/z$]

Temperature (°F.)	z = 8	z = 9	z = 10	z = 11	z = 12
179	0.0002	0.0006	0.0013	0.0023	0.0038
178	0.0003	0.0008	0.0016	0.0029	0.0046
176	0.0006	0.0013	0.0025	0.0043	0.0068
175	0.0008	0.0017	0.0032	0.0053	0.0083
174	0.0010	0.0022	0.0040	0.0066	0.0100
172	0.0018	0.0036	0.0063	0.0100	0.0147
170	0.0032	0.0060	0.0100	0.0152	0.0215
168	0.0056	0.0100	0.0159	0.0231	0.0316
166	0.0100	0.0167	0.0251	0.0351	0.0464
165	0.0133	0.0215	0.0316	0.0433	0.0562
164	0.0178	0.0278	0.0398	0.0534	0.0681
162	0.0316	0.0464	0.0631	0.0811	0.1000
160	0.0562	0.0774	0.1000	0.1233	0.1468
158	0.1000	0.1292	0.1585	0.1874	0.2154
156	0.1778	0.2154	0.2512	0.2848	0.3162
155	0.2371	0.2783	0.3162	0.3511	0.3831
154	0.3162	0.3594	0.3981	0.4329	0.4642
152	0.5623	0.5995	0.6310	0.6579	0.6813
150	1.000	1.000	1.000	1.000	1.000
148	1.778	1.668	1.585	1.520	1.468
146	3.162	2.783	2.512	2.310	2.154
145	4.217	3.594	3.162	2.848	2.610
144	5.623	4.642	3.981	3.511	3.162
142	10.00	7.743	6.310	5.337	4.642
140	17.78	12.92	10.00	8.111	6.813
138	31.62	21.54	15.85	12.33	10.00
136	56.23	35.94	25.12	18.74	14.68
135	74.99	46.42	31.62	23.10	17.78
134	100.00	59.95	39.81	28.48	21.54
132	177.80	100.00	63.10	43.29	31.62
130	316.20	166.80	100.00	65.79	46.42
128	562.30	278.30	158.50	100.00	68.13
126	1000.00	464.20	251.20	152.00	100.00
125	1334.00	599.50	316.20	187.40	121.20
124	1778.00	774.30	398.10	231.00	146.80
122	3162.00	1292.00	631.00	351.10	215.40
120	5623.00	2154.00	1000.00	533.70	316.20

staphylococci varied to give from 80,000 to 900 billion organisms per gram. In these experiments the tubes were processed at a number of temperatures in order to determine the slope of the thermal death time curve or z value.

The meats containing the suspended staphylococci with and without cure were then filled into sterile 10 x 75 mm. test tubes, sealed, and held for both 24 hours and 14 days at 6.1°C.(43°F.). These tubes were then processed in an oil bath at 65.8°C.(150.5°F.), controlled to within 0.17°C. (0.3°F.). After cooling, the three tubes (approximately one gram of meat each) at each processing level were aseptically transferred to a two-oz., screw-cap bottle containing 30 ml. (making 1 to 10 dilution) of salt broth and glass beads. These bottles were then placed in a mechanical

shaker, and when mixing was complete one ml. was removed and four-per cent salt-agar plates made and incubated for 48 hours at 37°C. Slides were prepared from typical colonies and checked under the microscope for characteristic morphology.

The data expressing the equivalent value of the thermal processes as F' are summarized (Tables 2 and 3). An F' value of one is a thermal process the equivalent of heating one minute at 150°F., assuming instantaneous heating and cooling and assuming a z value of 9°F. The data for Experiments 3 and 4 (Table 2), holding 24 hours at 43°F., no cure and with cure using 48-hour cultures, are comparable to the conditions of Stritar's experiments. The data are the reverse of those presented by Stritar since Stritar showed two and seven minutes at 66.1°C.(151°F.), respectively, for uncured and cured meat, whereas data (Table 2) show 10 and four minutes, respectively, at 150°F. There was some evidence of the greater thermal resistance of the organisms from the 14-day culture. Holding the tubes of inoculated meat for 24 hours and for 14 days at 43°F. before processing had little or no effect on thermal resistance within the errors of duplicating the experiments.

TABLE 2

F' Value for 10,000 per Gram of 48-Hour and 14-Day Salt-Broth Cultures of Staphylococci Suspended in Ham

Experiment	F' value			
	48-hour culture		14-day culture	
	Survived	Destroyed	Survived	Destroyed
1. Sterilized ham, without cure				
Held 24 hours 43°F.	2	4	6	8
Held 14 days 43°F.	....	Less than 1	2	3
2. Sterilized ham, with cure				
Held 24 hours 43°F.	3	4	5	10
Held 14 days 43°F.	4	5	5	10
3. Ham, aseptic technique, without cure				
Held 24 hours 43°F.	8	10	20	25
Held 14 days 43°F.	5	10	10	20
4. Ham, aseptic technique, with cure (sterilized)				
Held 24 hours 43°F.	2	4	2	4
Held 14 days 43°F.	3	4	....	Less than 1

The results of heating different numbers of 48-hour broth cultures of staphylococci in sterilized cured ham at different processing temperatures are shown (Table 3). The data show a general tendency for the process value for destruction (F') to increase as numbers increase. The z value or slope of the thermal death time curve is apparently unaffected and the average value is 9°F.

Thermal Death Time of Staphylococci in Meat When Grown in Meat: This portion of the experiment may logically be based upon the condition of the meat at the time of inoculation, namely, raw, pasteurized, or sterilized, and of course curing agents might or might not be present in each case. Further, the inoculated product might be incubated for 48 hours or 14 days.

In all phases of the experiment raw hams were cut up in an aseptic manner and the lean portion only used. The meats were used raw, pasteurized, or sterilized and sterilized curing agents were added as indicated. Two ml. of a 48-hour salt-broth suspension of *Staphylococcus* No. 184 were used as inoculum and the bottles were incubated for 48 hours at 37°C.

TABLE 3

Thermal Resistance of 48-Hour Salt-Broth Cultures of Staphylococci Suspended in Sterilized Cured Ham

Number of organisms	Condition	Corrected time in minutes[1] at					F'	z
		136°F.	139°F.	142°F.	145°F.	148°F.		
900 billion	Survived	324	150	61.9	25.1	15.0	....	....
	Destroyed	359	167	69.6	28.7	16.7	9.7	9.0
100 billion	Survived	287	117	69.6	25.1	......	....	....
	Destroyed	324	133	77.4	28.7	......	9.6	8.8
100 billion (check)	Survived	287	117	61.9	25.1	......	....	....
	Destroyed	324	133	69.6	28.7	......	8.4	8.8
10 billion	Survived	251	......	46.4	25.1	13.3	....	....
	Destroyed	287	......	54.2	28.7	15.0	9.1	9.5
4 billion	Survived	216	100	38.7	14.4	8.3	....	....
	Destroyed	251	117	46.4	17.9	10.0	5.4	8.3
80 thousand	Survived	144	83	38.7	14.4	8.3	....	....
	Destroyed	180	100	46.4	17.9	10.0	6.0	9.4

[1] Corrected for heating and cooling lag of three minutes.

TABLE 4

Typical Values for pH and Number of Organisms After Growing in Meat

Handling[1]	pH			Numbers of cocci[2]	
	Inoculated and incubated for			Inoculated and incubated for	
	Initial	48 hours	14 days	48 hours	14 days
Sterilized meat.....................................	5.92	5.82	6.10	500 million	100 million
Sterilized meat with cure....................	6.10	5.82	6.01	1 billion	1 billion
Pasteurized meat, aseptic technique..	5.92	5.70	6.81	1 billion	200 million
Pasteurized meat, aseptic technique, sterilized cure added........................	6.03	5.70	7.22	2 billion	2 billion
Fresh meat, aseptic technique.............	5.81	6.55	7.87	2 billion	100 thousand
Fresh meat, aseptic technique, sterilized cure added........................	5.57	5.70	6.84	600 million	100 million

[1] Same meat used on all experiments. Coccal forms on phenol red mannitol salt agar, none on fresh meat alone, and 10 per gram on fresh meat with sterilized cure added. [2] Using phenol red mannitol salt (7.5%) agar incubated at 37°C.(98.6°F.).

(98.6°F.). Grinding and mixing in an aseptic manner after incubation served to distribute the organisms uniformly throughout the mass. Typical pH values determined at the beginning and end of the growth periods as well as the numbers of organisms at those times are shown (Table 4). Counts were made using decimal serial dilution technique. In cases where definite numbers were used they were obtained by dilution with sterilized cured or uncured meat as the case might be. The meat was stuffed into

10 x 75 mm. tubes. The tubes were heat-sealed and immediately processed for different times at various temperatures and then cooled. The technique of end-point determination was varied somewhat from that previously described. Phenol red mannitol agar containing 7.5 per cent salt was used. Slides were prepared from typical colonies and checked under the microscope for characteristic morphology. The determination of the end point using sterilized meat was relatively simple. In the case of pasteurized meat other organisms were of course present. The same situation was true to a greater extent with fresh meat, cured and uncured. By using phenol red mannitol agar containing 7.5 per cent salt it was possible to repress the growth of all but coccal forms. However, in our opinion we were not able to differentiate between *Staphylococcus* No. 184 and any other staphylococcal forms of similar size. Since there were few if any coccal forms present, however, in the original meat (see footnote to Table 4) after a relatively heavy inoculation and incubation for the periods noted the coccal forms present would be expected to be largely *Staphylococcus* No. 184. Obviously owing to the lack of appropriate diagnostic methods it was not possible to check the identity of all the organisms isolated. At intervals the identity of the organism isolated, however, was confirmed by demonstration of enterotoxin formation in salt-broth cultures using susceptible human volunteers. Therefore it can be seen that in the cases of pasteurized and fresh meat both with and without cure the end point was rather difficult to determine although not impossible. The errors involved in the determination of the end point are greater and, as mentioned above, the presence of other staphylococcal forms which may have been isolated and listed as *Staphylococcus* No. 184 is not impossible. The experiments do indicate as the end point, however, the destruction of all coccal forms which grow on phenol red mannitol agar containing 7.5 per cent salt.

Experiments were made using the same lot of meat and handling all factors uniformly throughout. The data (Table 6) are from such an experiment excepting the values for six and nine billion organisms grown in sterilized meat with cure. Likewise all data (Table 7) are on the same lot of meat after sterilization. These data indicate that pH appears to be a relatively minor factor in affecting the thermal resistance compared with age and the substrate.

When the organisms grew in sterile meat with or without cure for 48 hours the thermal resistance was relatively low, namely destroyed at a maximum F' value of 13 as shown (Tables 5 and 7). However, when grown for 14 days the maximum values for destruction were an F' value of 130 as shown (Tables 6 and 7).

For pasteurized meat the maximum values for destruction were F' values of 100 and 8 for 48 hours and 14 days, respectively, as shown (Tables 5 and 6). These values are the reverse of those obtained for sterilized meat. Further, there is apparently an effect of the cure on the 14-day growth to lower the heat resistance which is not apparent on the 48-hour growth, however the data are insufficient to warrant any definite conclusions.

Unusual results were obtained on fresh meat as shown (Tables 5 and 6). For 48-hour growth the maximum F' value was 105. The values were high

TABLE 5

Thermal Death Time of Staphylococci Grown in Meat for 48 Hours

Handling	Organisms per gram	Condition	Corrected time in minutes at 136°F.	139°F.	142°F.	145°F.	148°F.	F'	z
Sterilized meat with cure	80 thousand	Survived	144	83	38.7	21.5	8.3	6.2	9.7
		Destroyed	179	100	46.5	25.2	9.4		
	4 million	Survived	215	120	38.7	14.3	8.3	7.6	9.4
		Destroyed	250	133	46.5	18.0	9.4		
	10 billion	Survived	250	150	46.5	25.2	13.3	8.6	9.4
		Destroyed	287	167	54.0	28.8	15.0		
	900 billion	Survived	323	150	62.0	25.2	15.0	9.5	9.0
		Destroyed	359	167	70.0	28.8	16.7		
			143.8°F.	150.5°F.	156.8°F.				
Pasteurized meat, aseptic technique	100 million	Survived	305	57.6	11.5			66	9.4
		Destroyed	319	62.1	12.4				
	300 million	Survived	147	21.3	3.5			26	8.1
		Destroyed	172	22.2	5.3				
	800 million	Survived	246	53.2	10.6			67	10.0
		Destroyed	270	62.1	12.4				
Pasteurized meat, aseptic technique, sterilized cure added	700 million	Survived	295	57.6	11.5			68	9.0
		Destroyed	319	62.1	12.4				
	800 million	Survived	467	84.2	17.7			100	9.1
		Destroyed	491	88.7	19.4				
	900 million	Survived	393	66.5	15.0			82	9.1
		Destroyed	418	71.0	15.9				
Fresh meat, aseptic technique	1 million	Survived	348	62.0	.....			71	9.4
		Destroyed	354	63.0	.....				
	600 million	Survived	295	48.8	12.4			63	9.5
		Destroyed	344	53.2	14.1				
	800 million	Survived	319	57.6	11.5			64	9.1
		Destroyed	344	62.0	12.4				
Fresh meat, aseptic technique, sterilized cure added	10 million	Survived	368	66.5	13.2			77	9.2
		Destroyed	393	71.0	14.1				
	2 billion	Survived	442	88.7	16.8			105	9.0
		Destroyed	467	97.6	17.7				
	60 billion	Survived	393	88.7	16.8			100	9.2
		Destroyed	417	97.6	17.7				

for both cured and uncured meat although there appeared to be a tendency for higher F' values on the cured meat. For 14-day growth the F' values were 11 and 120 for uncured and cured meat, respectively. The wide spread in F' values may be partly an effect of curing agents since it appears doubtful that it is due to the difference in number of organisms alone.

The data (Table 5) show that the z value or slope of the thermal death time curve is apparently unaffected by the variations in substrate and numbers.

TABLE 6

Thermal Death Time of Staphylococci Grown in Meat for 14 Days

Handling	Organisms per gram	pH	Thermal resistance		
			Time in minutes (corrected[1]) at 150.5°F.		F'
			Survived	Destroyed	Destruction
Sterilized meat..	100 million	6.10	35.5	43.5	49.0
Sterilized meat with cure....................	1 billion	6.01	106.5	115.3	130.0
	6 billion		106.5	115.3	130.0
	9 billion		97.6	106.5	120.0
Pasteurized meat, aseptic technique.	200 million	6.81	6.2	7.1	8.0
Pasteurized meat, aseptic technique, sterilized cure added.........................	2 billion	7.22	0.9	1.9	2.1
Fresh meat, aseptic technique............	100 thousand	7.87	8.9	9.8	11.1
Fresh meat, aseptic technique, sterilized cure added.........................	100 million	6.84	97.6	106.5	120.0

[1] Corrected for heating and cooling lag of three minutes.

TABLE 7

Thermal Death Time of Staphylococci Grown in Sterilized Meat

Handling	Incubated	pH	Number of staphylococci	Thermal resistance[1] F^1	
				Survived	Destroyed
Sterilized meat with cure....................	48 hours	4.05	100 million	12	13
	14 days	5.50	800 million	110	120
Sterilized meat....................................	48 hours	5.45	100 million	8	10
	14 days	6.46	90 million	120	130

[1] Determined at 150.5°F. Values equivalent to minutes at 150°F. assuming instantaneous heating and cooling.

Thermal Resistance of Staphylococci Suspended in Broth and Buffer: The thermal resistance of 48-hour and 14-day salt-broth cultures of *Staphylococcus* No. 184 suspended in salt broth and buffer solutions is of interest because the results may be compared with data in the literature. Thermal-resistance tests were made on 10,000 organisms per milliliter suspended in salt broth and buffer and the data are recorded (Table 8).

Growth of Staphylococci in Meat Products: The data reported are from individual unrelated experiments and not from an organized series.

They should be regarded as preliminary and incomplete and are presented merely for the record; the authors realize that further work is needed before valid conclusions can be drawn.

Growth in Meat: Growth was excellent in raw, pasteurized, and sterilized meat with and without cure at 37°C.(98.6°F.) in a 48-hour period. In the raw and pasteurized meat, with and without cure added, held longer than 48 hours at 37°C. there is apparently a simultaneous growth of other organisms normally present as well as the inoculated staphylococci. Through use of a selective media, such as phenol red mannitol agar containing 7.5 per cent salt, it is possible to determine the numbers of coccal forms. The data (Table 4) show that by 14 days of incubation there has been a decrease in numbers of staphylococci unless curing agents are present. The presence of the curing agents tends to exert a selective effect in favoring the growth of the salt-tolerant coccal forms and probably repressing to some extent the rod forms.

TABLE 8

Thermal Death Time of Staphylococci Suspended in Salt Broth and Buffer

10,000 organisms per ml. heated at 143.8°F. in	Thermal resistance F'			
	48-hour culture		14-day culture	
	Survived	Destroyed	Survived	Destroyed
Salt broth, pH 6.9	2	3	3	4
Buffer, pH 7.0	3	4	4	Above 4

Growth in Curing Pickles: The curing pickles used were made from a saturated brine diluted to 90° and 60° salometer. Sugar, sodium nitrate, and sodium nitrite were added in amounts normally used. One set was inoculated with 0.01 ml. of a salt-broth suspension of staphylococci containing 900 billion organisms per milliliter and the other set was pasteurized [heated 135 minutes at 73.9°C.(165°F.) in eight-oz. bottles], cooled, and then similarly treated. Bottles were incubated at 37, 20, and 0°C. (98.6, 68, and 32°F.) at times of 48 hours, one week, and two weeks, respectively, before the first examination and then held for another similar period and re-examined. Based on turbidimetric measurements there was no evidence of growth in any of the bottles.

Growth Below 10°C.(50°F.): Three experiments were made using salt broth held 14 days at 6.1°C.(43°F.) after inoculation and no increase in numbers could be determined within the limit of reproducibility of the counting technique. Eight-ounce bottles containing four ounces of salt broth were inoculated at room temperature with 48-hour broth cultures of staphylococci and held at room temperature for seven hours and then cooled to 43°F. in ice and held in a refrigerator at 43°F. for 10 days. No increase in numbers was noted. This experiment was repeated and the count into the refrigerator was 20 million and out of the refrigerator was 90 million. Five ml. of this culture after 10 days at 43°F. were taken by a susceptible human volunteer with negative results.

Warm pork-cheek meat trimmings (seven ounces in an eight-ounce sterile bottle) direct from slaughtered carcasses were inoculated with one

ml. of 48-hour, actively growing, salt-broth culture of staphylococci and left at room temperature for five hours and then placed in a refrigerator at 43°F. The count when placed in the refrigerator was one million and when removed 10 days later was 10 million. This experiment was repeated and counts were 20 million and 10 million into refrigerator and after 10 days at 43°F., respectively. A third repetition yielded data of 20 million and 200 million, respectively, for going into the refrigerator at 43°F. and after 10 days.

The lean portion was removed from a chilled fresh ham using aseptic technique insofar as possible, and after being ground in a sterile grinder the meat was at room temperature of 23.9°C.(75°F.). It was then inoculated by adding one ml. of an actively growing, 48-hour, salt-broth culture of *Staphylococcus* No. 184 to seven ounces of meat in a sterile, eight-oz., screw-cap bottle and was left at room temperature for five hours. At this time the count was 20 thousand. It was then placed at 43°F. and held for five days; there was no apparent increase in numbers using phenol red mannitol salt agar. Three grams of the meat treated as outlined were fed to a susceptible human volunteer with negative results.

TABLE 9

Thermal Death Time of Staphylococci in Meats After Holding for Five Days at 6.1°C.(43°F.)

Handling	Numbers of staphylococci		Thermal resistance F'	
	Into refrigerator at 43°F.	After 5 days at 43°F.	Survived	Destroyed
Sterilized meat...	100	1,000	3	4
Sterilized meat, sterilized cure added....................	100	10,000	2	3
Meat, aseptic technique, sterilized cure added......	1,000	100,000	5	6

Lean meat from a fresh ham was handled aseptically and sufficient 48-hour salt-broth culture of staphylococci added to give 1,000 organisms per gram. Cure was also added to one portion. The stuffed, sealed tubes were held at 43°F. for five days. No increase in numbers could be determined by standard counting technique. After heat-processing the usual method of determining end point of survival was used. In both portions with and without cure added the F' values were two survived and three destroyed.

Lean meat from a ham was handled aseptically and to one part curing agents were added. Another part was sterilized and still another part was sterilized and at the time of adding staphylococci sterilized curing agents were also added. Inoculations of a 14-day salt-broth culture of staphylococci were added and distributed throughout in an aseptic manner. After holding the stuffed, sealed tubes for five days at 43°F. counts and thermal death time determinations were made. The data are shown (Table 9) and indicate that there has been no significant growth on the basis of counts. There was also no significant difference in thermal death times for sterilized meat and fresh meat.

DISCUSSION AND SUMMARY

Staphylococci have been recognized as relatively heat resistant among the nonsporeforming bacteria. Topley and Wilson (1941) state that staphylococci are among the more resistant of the nonsporing organisms and that many are heat resistant in that they will withstand a temperature of 60°C.(140°F.) for half an hour. The same authors state that streptococci of the faecalis-lactis group are characteristically resistant to heat and most strains will withstand a temperature of 60°C. for 30 minutes or more. About the dysentery subgroup, they state that they are not specially resistant and are killed at a temperature of 55°C.(131°F.) for one hour. Yesair, Cameron, and Bohrer (1944) state that in the normal moist state micrococci were killed in 30 to 45 minutes at 55°C. These times when calculated to the F' basis are 0.2 to 0.3. The strain of *Staphylococcus* No. 184 used in this study was reported by Stritar (1941) to be the most heat resistant of 14 strains of food-poisoning origin. Stritar found that two minutes at 65.6°C.(150°F.) or an F' value of 2 were sufficient to destroy a concentration of one million organisms suspended in buffer at pH 7. Beamer and Tanner (1939) reported *Staphylococcus aureus* survived 13.8 minutes and was destroyed in 18.8 minutes at 65°C. (149°F.). The *S. aureus* used by Beamer and Tanner was Culture 42 from the University of Chicago isolated from a case of food poisoning, and 3.2 million cells were suspended in broth at pH 7.05. These values calculated to an F' basis are 10.3 and 16.6 for survival and destruction, respectively. Therefore, while agreement is not good, the order of the values is relative and distinctly lower than the unusually high values obtained in certain of the experiments reported in this paper using meat substrates. All data apparently support the conclusion that *Staphylococcus* should be considered a relatively heat-resistant organism among the nonsporeforming group. The heat resistance of the particular strain used in this study is within the normal range of values obtained by other investigators when suspended in broth or buffer solutions but it is not necessarily the most heat-resistant strain.

It is suggested that staphylococci might be suitable as a test organism in a study of the lethality of pasteurization processes. Pasteurization has been defined by Buchanan and Fulmer (1930) as "a term used to designate the process of heating a food material to such a temperature and for such period of time as will destroy certain types of undesirable organisms, or in some cases reduce their numbers below a certain desired minimum." It is also suggested that the arbitrary value F' might be a convenient way to express the lethality of a pasteurization process so that no further information would be required to describe the process except the value of z, which might be known, determined, or assumed.

The comments of Dack (1943) are very interesting and pertinent, namely, that enterotoxin production is a function of growth and that enterotoxin could be demonstrated in cultures after 12 hours at 37°C. (98.6°F.) and three days at 18°C.(64.4°F.) but not in cultures grown at shorter periods or for seven and three days at 9°C.(48.2°F.) and 15°C. (59°F.), respectively, or after four weeks at 4°C.(39.2°F.) and 6.7°C.

(44.1°F.). Also important are the comments of Dack that certain foods, such as canned salmon, may support growth without production of enterotoxin and that meat products allow food-poisoning staphylococci to grow well and to produce enterotoxin.

Comments by Jensen (1945) are also of interest and are as follows: "Staphylococci are killed at pasteurizing temperatures—61.7°C. (143°F.)—for 30 minutes. The *Staphylococcus* does not form poisons unless it grows, and it only grows well at temperatures from 21.1 to 40.6°C. (70 to 105°F.). It requires four to eight hours of steady growth (logarithmic phase) in a rich food medium at 37.2°C. (99°F.) to produce enough toxin to elicit symptoms (vomiting and diarrhea) in man or monkeys when the food or broth is swallowed."

Comments by Halvorson (1944) are of interest in connection with his statement that "the heat resistance will vary with the composition of the medium and with the past history of the organisms." He also states: "Vigorous growth of the organism appears to be necessary for toxin formation, since food heavily contaminated with staphylococci will not become toxic if it is stored in an icebox. If it is left at room temperature for a few hours, however, it will be toxic. About all that can be said from our present state of knowledge is that any food that will support the growth of staphylococci is likely to become toxic if left at a favorable temperature."

In this investigation the thermal death times found for the suspension of staphylococci in meat, both sterilized and raw handled in an aseptic manner, were of the order of those found by Stritar. The effect of cure noted in this investigation was the reverse of that noted by Stritar, although perhaps conclusions are not fully warranted on the basis of the observed variations because of the large possible error in replication. The order of these thermal death times was not too far from agreement with the values found in broth and buffer indicating no especial effect of the meat substrate upon the thermal resistance. There was a tendency for the resistance of cultures grown for 14 days to be somewhat higher than those grown for 48 hours. Holding the sealed tubes at 43°F. for as long as 14 days before processing had no apparent effect on the thermal death time. The F′ values for destruction increased as the number of organisms increased while the z value remained constant at approximately 9°F.

In order to realize experimental conditions more closely comparing with commercial practice, the artificially inoculated broth cultures of staphylococci were permitted to grow in meat and the thermal death time was then determined. There were three general possibilities, namely, growing in sterilized, pasteurized, and fresh meat, and of course cure might be present or absent. There were some general conclusions that might be drawn. When there was an increase in numbers of bacteria present there was a related increase in F′ value for destruction. Further, the lowest thermal death times found, with one exception, were higher than for the suspensions of broth cultures in meat. For all conditions used the slope of the thermal death time curve was shown to have an average value of 9.2°F. and not to be affected by changes in numbers or substrate.

For the purposes of discussion the results may be divided into two major groups based on whether the staphylococci have been allowed to grow for 48 hours or 14 days at 37°C. In the experiments on 48-hour growth in sterilized meat with and without cure the F' values for destruction range from 6.2 to 13 and no apparent conclusion is warranted with regard to the effect of curing agents. In pasteurized meat the thermal resistance is greatly increased and the F' values for destruction ranged from 26 to 100. Here again no conclusion is warranted as to the possible effect of curing agents upon the thermal resistance. In the experiments conducted on fresh meat the same conclusions can be made as on pasteurized meat. In all of these experiments there is no apparent change in the z value and therefore it can be concluded that a change of numbers and substrate as indicated in the various experiments has no effect on the z value.

In the experiments in which the staphylococci were allowed to grow in the meat for 14 days there were some deviations from the results obtained for the 48-hour incubation. For sterilized meat the F' values were extremely high when cure was added and moderately high without the cure, but inasmuch as the results from only two experiments are available it is doubtful if a conclusion as to the effect of the curing agents is warranted. In experiments conducted on pasteurized meat the thermal death times were relatively low and in the range of reported values for the destruction of *Staphylococcus* in broth or buffer. Here again no conclusion is warranted as to the possible effect of curing agents. In those experiments in which raw meat was used a very distinct difference was noted between cured and uncured product. The one with curing agents had a thermal resistance almost ten times as great as the one without curing agents and it appears doubtful that the difference in numbers could have been responsible for the tremendous difference in thermal resistance. The level of thermal resistance for the fresh meat with cure is of the same order as that of sterilized meat with cure, while the level of thermal resistance of fresh meat without cure is of the order of that in pasteurized meat.

Experiments were made using the same lot of meat throughout and the object of the experiments was to obtain a comparison of the effect of 48-hour and 14-day incubations. The 48-hour samples were placed under refrigeration at the end of the 48 hours and the experiment conducted when the 14-day incubation period was over. In sterilized meat with cure there is an increase of almost tenfold in thermal resistance apparently owing largely to the longer period of incubation, since the numbers are not sufficiently different to account for it. In the sterilized meat without cure there is a similar disparity between the thermal resistance for 48 hours and 14 days, and the numbers are almost identical.

We offer no mechanism or suggestions to account for the difference in thermal resistance as recorded in the various experiments. The results are reported with the thought of stimulating further investigation. For various reasons this line of investigation can be carried no further at the present time but certainly the implications are that the results to be obtained by further investigation would be worth while.

The data on the growth of staphylococci in meat products and pickles are incomplete and consist largely of individual, unrelated experiments. Staphylococci grow well in raw, pasteurized, and sterilized meat with and without cure when incubated 48 hours at 37°C. When incubated for longer periods, such as 14 days, in raw and pasteurized meat with and without cure there is often found a decrease in numbers at 14 days. A number of factors may be responsible for this decrease in numbers but it is interesting to note that the numbers were never greater after the 14-day incubation and in many cases were lower. As pointed out in another portion of this paper, the methods of isolation used were not sufficiently accurate so that it was certain that all coccal forms isolated were *Staphylococcus* No. 184. The only conclusion warranted is that within the limits of the experiments made actively growing staphylococci inoculated in meat did not grow and produce enterotoxin when held for as long as two weeks at 43°F., as indicated by several checks on ingestion of product by susceptible human volunteers. This observation is in agreement with the observations of Dack (1941).

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RETENTION OF SOME VITAMINS OF THE B-COMPLEX IN BEEF DURING COOKING [1]

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The extent to which cooking may alter the nutritive value of foods has been the subject of many investigations in recent years. A few of these have been concerned with the effects of cooking on the retention of vitamins of the B-complex in meat. McIntire, Schweigert, Henderson, and Elvehjem (1943); McIntire, Schweigert, and Elvehjem (1943); McIntire, Schweigert, Herbst, and Elvehjem (1944); and Schweigert, McIntire, and Elvehjem (1943) have determined the retention of thiamin, riboflavin, and nicotinic acid during cooking of veal, lamb, pork, variety meats, and cured pork hams. Cover, McLaren, and Pearson (1944) determined the retention of thiamin, riboflavin, nicotinic acid, and pantothenic acid in rare and well-done rib roasts of beef. These investigations indicate that meat in general retains a high percentage of the vitamins of the B-complex during cooking, but that there is some heat destruction of thiamin and pantothenic acid. Since many of the retail cuts of beef require moist heat and longer cooking periods than those used for the rib roasts in Cover's study (1944), there is further need to demonstrate the extent to which different methods of cooking beef may affect the retention of the B-vitamins.

The present investigation was designed to study the retention of nicotinic acid and pantothenic acid when beef was braised, broiled, and fried under standardized conditions representative of good home-cooking procedures. In the experiments on fried beef liver the retentions of thiamin and riboflavin were also included.

EXPERIMENTAL PROCEDURE

Braising: Pot roasts were used for the braising studies. Heel-of-round was chosen to represent chunky, boneless cuts and chuck to represent thinner cuts with more surface area. Four pot roasts of each cut were used. The heel-of-round roasts weighed an average of 3.2 pounds and the chuck roasts 3.5 pounds before cooking. The heel-of-round roasts were cooked in a cast-aluminum pan, and the chuck roasts in a cast-iron pan. Both pans were provided with self-basting covers.

The cooking was started when the internal temperature of the meat was 10 to 12°C. (50 to 53.6°F.), by searing for 15 minutes in fat trimmed from the cut, using a full gas flame. After searing, a meat thermometer was inserted into the center of the meat, 25 ml. of water were added, and the pan was immediately covered. Two thermometers fitted into a cork were

inserted through an opening in the cover, one to record the temperature of the air and the other the temperature of the liquid surrounding the meat. The third thermometer, which recorded the internal temperature of the meat, projected through another very small opening in the cover. Both types of pot roasts were cooked to an internal temperature of 93°C. (199.4°F.). All temperatures were recorded at approximately 20-minute intervals during the cooking process and the flame was adjusted to keep the liquid below the boiling point until during the last 30 minutes of cooking.

It was not possible to obtain paired roasts at the time this phase of the study was done. Therefore, five- to six-pound roasts were purchased in the retail market, and for the raw samples, slices were cut from each surface in an attempt to represent all muscles. These were ground together to obtain samples for the moisture, fat, and vitamin content of the raw meat. This method of obtaining a raw sample was considered adequate, since the vitamin retention was calculated from the total, fat-free, dry weight of the raw meat, cooked meat, and drippings, as described by Hinman, Tucker, Jans, and Halliday (1946). Therefore, it was not necessary to have the moisture and fat equally distributed in the part used for raw sampling and that taken for cooking.

Broiling: For the broiling studies four pairs of sirloin steaks from a U. S. Choice beef were used. All steaks were quick-frozen in the packing house the day the beef was cut up and were thawed overnight at refrigerator temperature before using. Alternate steaks from the right and left sides were cooked and the corresponding steak from the opposite side was taken for the raw analyses. The steaks were 3.5 to 4.0 cm. thick and were broiled to an internal temperature of 58°C.(136.4°F.) (rare) in the broiling oven of a gas range. The temperature directly above the meat was maintained at about 170°C.(338°F.) according to the standard procedure described in "Meat and Meat Cookery" (1942).

Frying: For the frying studies beef liver and round steaks were used. The liver was purchased in the open market. It was sliced one-fourth inch thick and alternate slices were taken for the raw and cooked analyses. It was fried one and one-half minutes on each side in an iron skillet with approximately 40 gm. of lard. A thermometer was suspended above the center of the skillet so that the bulb just touched the surface. Cooking was started when the thermometer registered 150°C. and the flame was adjusted to maintain a temperature between 120 and 150°C.(248 and 302°F.). In preliminary cooking tests this method was judged as giving a highly desirable product by workers in the laboratory.

Four pairs of round steaks were obtained from the same beef described under "Broiling" and were handled in the same manner. The steaks were approximately two centimeters thick and were pounded on each side with a meat tenderizer before frying. One and one-half minutes' pounding on each side was required to render the steaks sufficiently tender to cook by frying. After pounding, the steak was cut into individual servings and approximately one pound was fried at a time, cooking two minutes on each side. The cooking was started at 160°C.(320°F.), with the thermometer located as described for liver. A full gas flame was used throughout the

cooking period, and after a slight initial rise to around 168°C.(334.4°F.) the temperature registered on the thermometer dropped slowly throughout the cooking period, usually to about 115°C.(239°F.).

Cooking Losses: The weight losses on cooking were determined in all cases. The raw meat was weighed just before cooking was started. At the end of the cooking period the meat with its accumulated drippings was immediately weighed in the cooking pan to determine the volatile losses. The meat was then removed from the pan and the weight of the drippings determined. The sum of the volatile and drippings losses gave the total cooking losses.

Drippings from all methods of cooking were retained and analyzed for moisture and fat as well as vitamin content.

Nicotinic Acid: The nicotinic acid determinations were carried out by the microbiological method, using the basal medium of Krehl, Strong, and Elvehjem (1943) except that pyridoxine was increased to 0.2 p.p.m. and biotin to 0.02 p.p.m. A slight tendency to drift was eliminated by these increases.

Pantothenic Acid: Pantothenic acid was determined microbiologically using a 24-hour growth period with *Lactobacillus arabinosis* and a basal medium described by Wilder (1944). With this method the standard curve was run at 10 levels, using a solution containing 0.05 microgram of calcium pantothenate per milliliter. The samples were run in duplicate at five levels and gave values which frequently agreed within six per cent at all levels. In all but three of 120 assays used in this study the values at all levels agreed within 20 per cent. In these three cases the pantothenic acid content was calculated by averaging three levels within 20 per cent agreement.

Although Skeggs and Wright (1944) have reported that the growth of *Lactobacillus arabinosis* is stimulated by fatty acids, there was no tendency to drift observed in these assays. Ether extraction was not used in the preparation of the samples since preliminary tests on raw and cooked beef, with and without ether extraction, gave almost identical values.

The standard recoveries, carried out routinely with each set-up, ranged from 88 to 115 per cent for all determinations, except in the assays of two samples of drippings with which average recoveries of 118 and 126 per cent were obtained. This range of recoveries was more satisfactory than several recovery values obtained in preliminary tests using *Lactobacillus casei* with the basal medium of Neal and Strong (1943), modified by the use of synthetic vitamins instead of Vitab. These findings were an important factor in the choice of method for the pantothenic acid assays.

Thiamin and Riboflavin: The determinations of thiamin and riboflavin in liver were carried out fluorometrically. Thiamin was determined by the method of Hennessy and Cerecedo (1939), using the double adsorption technique of Conner and Straub (1941) to include riboflavin. The modifications employed with these methods were those described by Hinman *et al.* (1944, 1946).

Moisture and Fat: In order to determine the fat-free, dry residues for the calculation of the vitamin retentions during cooking, moisture and fat

determinations were carried out on all samples of raw meat, cooked meat, and drippings, according to the procedure described by Hinman *et al.* (1946).

Preparation of Samples: The meat was prepared for sampling by grinding twice with a Kitchen Aid meat grinder using the fine plate. It was mixed for one minute at second speed after each grinding and its total weight determined just before samples were withdrawn. Fifty-gram samples of the ground meat were weighed into tared Waring blendor cups and blended with N/30 HCl. For the nicotinic acid and pantothenic acid determinations, aliquots of the blends were digested for 48 hours at 37°C. (98.6°F.) under toluene, using a mixture of equal parts of takadiastase and papain. The digests contained a 1:10 ratio of enzyme to meat (dry weight) and were buffered at pH 4.7. A preliminary test indicated that enzymic digestion rather than autoclaving with acid gave maximum liberation of nicotinic acid. Waisman, Henderson, McIntire, and Elvehjem (1942) demonstrated the necessity of using enzymic digestion for complete liberation of pantothenic acid from both raw and cooked muscle tissue, and suggested that liquefaction of the denatured proteins might be an important phase of the enzymatic action. Cheldelin and Williams (1942) showed that a combination of takadiastase and papain was highly efficient in extracting both nicotinic and pantothenic acids from animal tissue. After 48 hours' digestion the samples were heated on a steam bath for 15 minutes to precipitate soluble proteins. The cooled digests were made up to volume, and filtered through Whatman No. 40 filter paper. The filtrates were diluted as required for the vitamin assays.

For the determinations of thiamin and riboflavin in liver, aliquots of the blends were extracted for one hour with mechanical stirring in N/10 H_2SO_4 at 70 to 75°C.(158 to 167°F.). After cooling the extracts were digested with Clarase for 18 hours at 50°C.(122°F.) in a pH 4.3-4.5 medium.

To obtain samples of the drippings, the total quantity of known weight was heated to 60°C.(140°F.) in a covered bottle, shaken, and aliquots immediately transferred with a wide-mouthed pipette. The weighed aliquots were digested as described for muscle samples prior to vitamin determinations. It was necessary to homogenize the drippings from the fried and broiled meats, by blending 30 seconds in a Waring blendor before sampling.

<div align="center">RESULTS</div>

The weight losses on cooking (Table 1) show that, as would be expected, braising, a moist-heat method which required on the average from 40 to 48 minutes per pound, gave greater cooking losses than the dry-heat methods, broiling and frying. The losses during braising are accounted for by the increased amount of drippings.

The retentions of nicotinic and pantothenic acids are summarized (Table 2); in the case of nicotinic acid there was no marked difference in the total cooking retention with any method of cooking. The total average retentions for all cuts with all methods of cooking ranged from 88 to 96 per cent. If the amounts retained in the meat and recovered in

the drippings are compared, however, a distinct difference is observed between braising, on the one hand, and broiling and frying, on the other. In the braised cuts, where there was an increased amount of drippings, there was about twice as much nicotinic acid extracted from the meat. Approximately one-half of the original nicotinic acid was retained in the braised meat and about one-third was recovered in the drippings. In the broiled and fried cuts about four-fifths of the nicotinic acid was retained in the meat, and less than one-fifth was extracted into the drippings. There appeared to be little if any heat destruction of nicotinic acid, with the latter methods of cooking.

TABLE 1

Summary Cooking Data [1]

Method of cooking	Weight	Time per pound	Cooking losses					
			Volatile		Drippings		Total	
			Range	Av.	Range	Av.	Range	Av.
	lb.	min.	pct.	pct.	pct.	pct.	pct.	pct.
Braising								
Heel-of-round........	3.2	48	5-14	8	23-31	28	33-38	37
Chuck.....................	3.5	40	9-21	13	17-26	22	33-39	35
Broiling								
Sirloin....................	4.2	7	9-16	12	6-11	8	15-26	19
Frying								
Round....................	1.9	4	13-18	16	4- 6	5	19-22	21
Liver......................	1.1	6	4-14	11	....	....	....	....

[1]Average of three cookings of fried liver; average of four cookings of other cuts of meat.

TABLE 3

Retention of Thiamin and Riboflavin in Fried Beef Liver [1]

Vitamin	Retention					
	In liver		In drippings		Total	
	Range	Av.	Range	Av.	Range	Av.
	pct.	pct.	pct.	pct.	pct.	pct.
Thiamin..	81-89	83	2-8	6	87-91	89
Riboflavin..	89-102	96	2-8	6	98-105	102

[1]Average of four cooking tests for thiamin and riboflavin in liver and for thiamin in drippings; average of three determinations for riboflavin in drippings and total.

In the case of pantothenic acid, braising likewise caused a marked leaching of the vitamin from the meat, but in addition there was also some heat destruction. Thus, with the heel-of-round pot roasts, where the total cooking time averaged 48 minutes per pound, the total cooking retention of pantothenic acid averaged 72 per cent, while the chuck pot roasts, with an average cooking time of 40 minutes per pound, gave an average total cooking retention of 81 per cent. Application of the t-test to these two groups indicates that the difference is significant. The highest average total retention of pantothenic acid, 93 per cent, was obtained with broiled sirloin steaks cooked rare. While the fried steaks and liver had lower retentions on the average than the broiled steaks, the difference is statistically significant only in the case of the fried liver. Since the liver was sliced so thin, it might be surmised that the greater destruction of

TABLE 2

Effect of Three Methods of Cooking on Retention of Nicotinic and Pantothenic Acids in Beef

Method of cooking[1]	Time per pound	Retention of nicotinic acid						Retention of pantothenic acid					
		In meat		In drippings		In meat plus drippings		In meat		In drippings		In meat plus drippings	
		Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.
	min.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.
Braising													
Heel-of-round	48	50-62	57	29-39	35	84-99	92	45-55	49	17-29	23	70-74	72
Chuck	40	51-60	54	30-37	34	84-97	88	50-59	53	25-30	28	76-88	81
Broiling													
Sirloin	7	74-88	80	11-19	14	92-99	94	73-96	83	9-11	10	84-105	93
Frying													
Round	4	75-85	81	10-18	15	85-100	96	68-81	74	9-12	11	77-93	84
Liver	6	82-85	84	2- 8	6	87-92	90	62-81	70	2- 8	6	70-87	75

[1] Four cookings of each cut of meat.

TABLE 4

Nicotinic and Pantothenic Acid Contents of Beef Cuts
(Micrograms per gram moist weight)

Cut[1]	Method of cooking	Nicotinic acid				Pantothenic acid[2]			
		Raw		Cooked		Raw		Cooked	
		Range	Av.	Range	Av.	Range	Av.	Range	Av.
Heel-of-round (Commercial)	Braising	53-70	59	45-58	54	5.5-5.8	5.6	4.3-4.7	4.4
Chuck (ungraded)	Braising	38-41	39	32-36	33	6.1-8.8	7.1	4.8-8.2	6.0
Round (Choice)	Frying	47-62	54	45-71	59	3.8-5.3	4.7	4.0-5.0	4.7
Sirloin (Choice)	Broiling	40-45	43	44-47	45	4.1-5.9	4.9	4.4-6.1	5.2
Liver	Frying	131-158	149	133-178	154	74-92	82.0	63-78	71.0

[1] Four samples of each cut. [2] Expressed as calcium pantothenate.

pantothenic acid was probably due to the increased surface per pound of meat exposed to the heat rather than the length of the cooking period. With these dry-heat methods only about one-tenth of the pantothenic acid was recovered in the drippings, but with braising about one-fourth of the original pantothenic acid was contained in the drippings.

The results on retention of thiamin and riboflavin in fried beef liver (Table 3) indicate that riboflavin is completely retained during frying, although a slight amount is sometimes transferred to the drippings. With thiamin however, there is a slight loss averaging around 10 per cent.

The nicotinic acid and pantothenic acid contents of the various cuts, both raw and as cooked, are summarized (Table 4).

DISCUSSION OF RESULTS

Nicotinic Acid: The results on the retention of nicotinic acid during cooking are in good agreement with those of McIntire *et al.* (1943, 1943, and 1944) for veal and lamb, pork, and variety meats. With all methods of cooking they obtained total average retentions of nicotinic acid ranging from 90 to 100 per cent. Thus they reported "very little difference in the total vitamin retention in braising, broiling, and frying" but they too found that braising caused considerable leaching of nicotinic acid from the meat. They obtained about 65 per cent retention of nicotinic acid in braised pork-loin samples; an average of 61 per cent in braised cuts of lamb and veal; and from 50 to 60 per cent in braised liver, heart, and tongue, whereas when pork loin was roasted or broiled, the nicotinic acid retained in the meat ranged from 75 to 90 per cent. Their broiled and roasted lamb and veal retained an average of 80 per cent and broiled liver 85 per cent of the original nicotinic acid. Schweigert *et al.* (1943) also obtained a 79-per cent retention of nicotinic acid in roasted cured ham, and 85 per cent retention in fried-ham slices, with total retentions of 96 per cent in both cases.

All of these results, as well as the ones obtained in this study, are slightly higher than those reported by Cover *et al.* (1944) for rib roast of beef, cooked rare, where the retention of nicotinic acid averaged only 75 per cent in the meat with an additional four per cent in the drippings. However, they too obtained total retentions of 94 to 101 per cent in five of six rib roasts of beef, cooked well done.

The nicotinic acid content of the various cuts of raw beef muscle determined in this study ranged from 38 to 70 μg. per gm. This is in good agreement with the range of 39 to 60 μg. per gm. reported by Cover *et al.* (1944) for rib of beef of Commercial and Choice grades. It is a somewhat wider range than the 45 to 46 μg. per gm. reported by Cheldelin and Williams (1942) for five samples of beef round. The nicotinic acid in four samples of raw beef liver used in these experiments ranged from 131 to 158 μg. per gm. These results compare favorably with the values of 120 μg. per gm. reported by Cheldelin and Williams (1942) for one sample of beef liver, and 135 μg. per gm. determined by McIntire *et al.* (1944).

Pantothenic Acid: Very few studies of the retention of pantothenic acid in meat have been reported. Cover *et al.* (1944) found an average total retention of 93 per cent for rib roast of beef cooked rare which agrees with

the value 93 per cent, reported here for sirloin steak broiled rare. They obtained a total retention of 77 per cent for well-done rib roast with 75 per cent retained in the meat itself. The values of 72 and 81 per cent, respectively, for braised heel-of-round and chuck determined in this study are of the same order, however the braised cuts had only about 50 per cent of the original retained in the meat.

In 1940, Waisman and others reported "approximately 30 per cent" decrease of pantothenic acid in two samples of beef round after frying. They gave no figure for drippings. However, the retentions of 74 and 70 per cent, respectively, obtained here in fried beef round and liver would seem to be in good agreement.

The pantothenic acid content of the various beef muscles studied ranged from 3.8 to 8.8 μg. per gm. This is a somewhat wider range than the 3.8 to 4.7 value reported by Cover et al. (1944) for Commercial rib of beef. The average values of 5.6 obtained on heel-of-round, and 4.7 on round steak are considerably lower than the values of seven to 13 μg. per gm. reported by Waisman et al. (1942) for beef round. For beef liver, the values 74 to 92 μg. per gm. are in agreement with the values 55 to 92 μg. per gm. reported by Cheldelin and Williams (1942) and slightly higher than the values 44 to 88 μg. per gm. reported by Waisman et al. (1942).

SUMMARY

The effects of cooking on the nicotinic acid and pantothenic acid contents of beef pot roasts, fried round steaks, broiled sirloin steaks, and fried beef liver were determined. In the case of liver, thiamin and riboflavin retentions were also studied.

The results with nicotinic acid indicate good total retentions in meat and drippings, averaging 92 per cent for all cuts with all methods of cooking. However, braising extracted about one-third of the nicotinic acid into the drippings.

Pantothenic acid was retained to the greatest extent, 93 per cent in sirloin steak broiled rare. Fried round steak retained 85 per cent and fried liver 75 per cent. All of these include small amounts, six to 11 per cent in the drippings. Braised heel-of-round retained 72 per cent including 23 per cent recovered in the drippings, while braised chuck retained 81 per cent, with 28 per cent in the drippings.

Beef liver retained an average of 89 per cent thiamin and 102 per cent riboflavin after frying, including six per cent recovered in the drippings in both cases.

The nicotinic acid content of raw beef muscle ranged from 38 to 70 μg. per gm. and that of four samples of beef liver, from 131 to 158 μg. per gm.

The pantothenic acid content of raw beef muscle ranged from 38 to 88 μg. per gm. and that of four samples of beef liver from 74 to 92 μg. per gm.

Raw beef liver contained from 2.5 to 3.4 μg. per gm. of thiamin and from 29 to 32 μg. per gm. of riboflavin.

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EFFECT OF WEATHER, VARIETY, AND LOCATION UPON THIAMIN CONTENT OF SOME KANSAS-GROWN WHEATS [1]

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Numerous extensive investigations have been made studying the effect of environment upon the chemical constituents of the wheat berry, but no extensive work has been done in studying the effect of environment upon vitamin content of the wheat berry. It was to study the effect of environment on the thiamin content of wheat and the relationship of both variety and location on the average thiamin content of some Kansas hard red winter wheat varieties that the present investigation was undertaken. Since flours were available for each respective wheat sample, the thiamin content of each flour was determined in order to give a complete story of the effect of both variety and location on the thiamin content of wheat and flour.

Nordgren and Andrews (1941) and Conner and Straub (1941) have shown that both variety and environment are highly significant in affecting the thiamin contents of winter wheats. Recently Whitney, Herren, and Westerman (1945) have presented data showing that variety influences the thiamin content of wheat. Hunt (1927), in his work on the effect of fertilizers, has indicated that acid phosphate alone or in a complete fertilizer with potassium chloride and sodium nitrate produced wheat with the highest vitamin B content. Leong (1939) found that the thiamin potency of wheat was not influenced by soil treatment.

EXPERIMENTAL PROCEDURE

All the samples used in the present investigation were grown at various locations in Kansas as shown in the precipitation map (Fig. 1). Seven varieties were used as follows: Turkey, Blackhull, Tenmarq, Nebred, Chiefkan, Kawvale, and Clarkan. Samples were available for the years 1941 and 1942 from Tribune, Colby,. Garden City, Meade, Dodge City, Hays, Hutchinson, Kingman, Manhattan, Thayer, and Columbus. The varieties called Turkey, Blackhull, and Tenmarq were grown at all 11 stations; Nebred and Chiefkan were grown at all except Thayer and Columbus; and Kawvale and Clarkan, the two soft wheats, were grown at Manhattan, Thayer, and Columbus. The locations represented some extreme climatic conditions prevailing in Kansas, as indicated (Fig. 1).

The wheat and flour samples were stored in airtight jars in a refrigerated room at approximately 4.4°C.(40°F.) until thiamin determinations
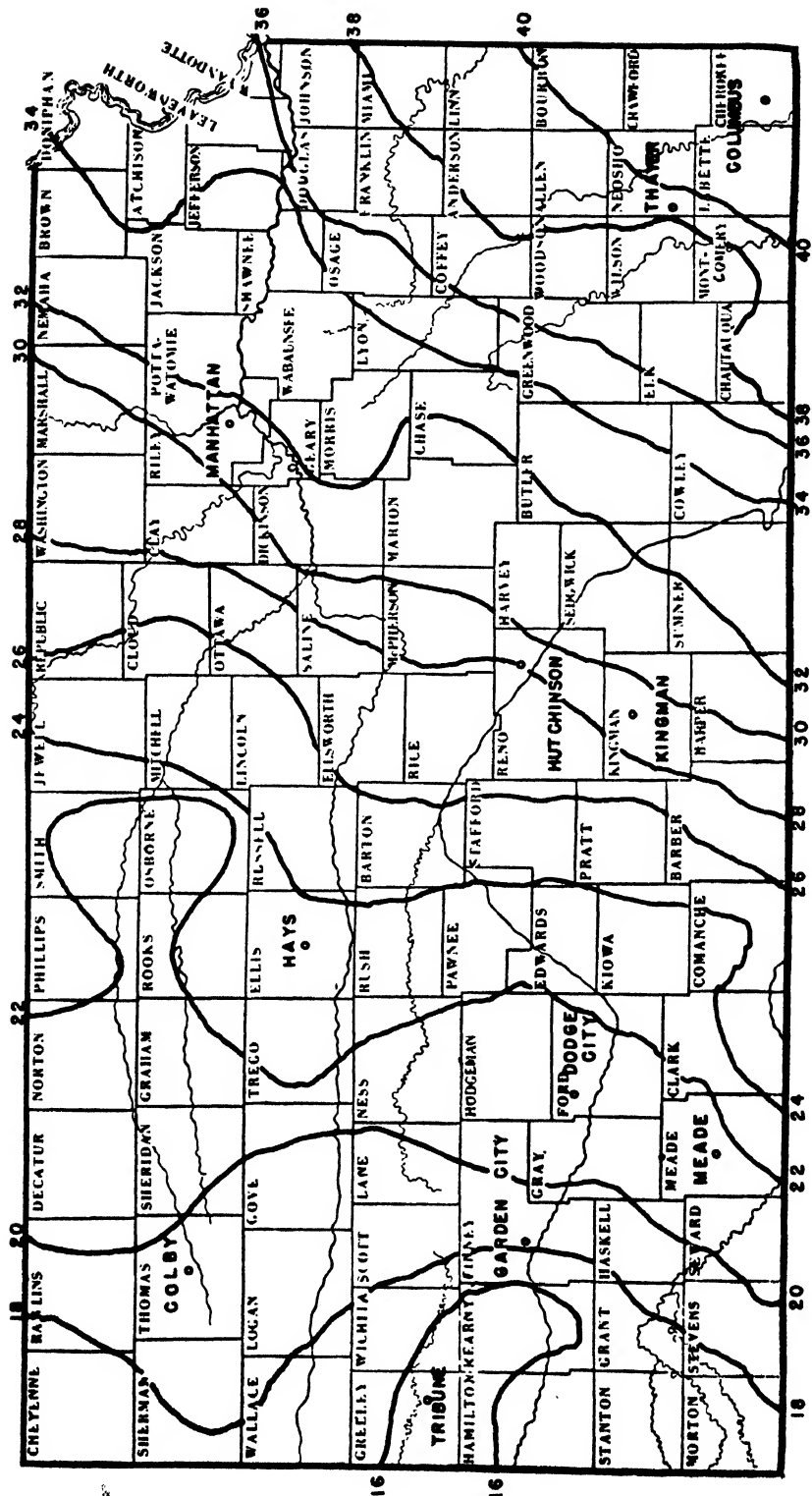
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Fig. 1. Precipitation areas in Kansas (inches).

were made. The storage period for any one sample did not exceed two months. Before the wheat was placed under refrigeration, it was passed through a Eureka cleaner to remove foreign material. The samples were finely ground at the time of analysis in a Hobart grinder, Model 275, to pass a 32-W screen.

All wheat samples were milled on an Allis-Chalmers experimental mill to an ash content of approximately 0.42 per cent. The milling process consisted of four breaks and seven reductions. In blending each sample the fourth-break and low-grade flour streams were used to make the blend approximately 0.42 per cent ash. The flours thus prepared represented patent flours with an average extraction of 88 to 90 per cent.

Thiamin content was determined by Hennessy's (1942) thiochrome method. Duplicate determinations agreeing within five per cent were made on all samples.

WEATHER CONDITIONS

Daily rainfall and daily maximum and minimum temperatures were obtained from April 15 to the date the wheat was ripe. April 15 was considered to be sufficiently early in the life of the plant to start collecting weather data to supply adequate information for an investigation of this type.

In order to study the influence of rainfall and temperature upon the thiamin content of wheat, the daily weather data were condensed into three periods from the date of ripening *back* to April 15. The three periods were called "before, during and after heading" (Tables 1 and 2).

The weather conditions for 1941 and 1942 were quite different, and it appears from the results that the growing conditions of 1942 were more favorable for thiamin production than were those of the preceding season. The 1941 season had sufficient precipitation throughout the time preceding the heading of the wheat, and during the heading stages rains were so plentiful that almost twice as much fell during this time as during the same period of growth in 1942. After the heading period rains were frequent and plentiful throughout the ripening stages, and the weather became increasingly warmer as the ripening progressed. The 1942 season preceding the heading stage was characterized by a scarcity of moisture which lasted until after heading. There was approximately twice as much precipitation during the 1941 heading period as during the heading period for 1942.

Rains during 1942 were more frequent and plentiful after heading than during the preceding year. In 1942 warm weather followed the heading stage, but cool weather prevailed during the late stages of ripening.

VARIETY, LOCATION, AND SEASON

When the data were analyzed statistically, it was found that there existed a significant difference between both varieties and locations as shown by analysis of variance. This is evident from the averaged chemical analysis data (Tables 3 and 4). For the 1941 crop, Turkey was the variety highest in thiamin content and, except for Tenmarq, it was significantly higher than all other varieties. The wheats from Tribune and Garden City were not significantly different but were significantly higher in thiamin

TABLE 1

Precipitation and Temperature for 1941, in Three Periods From April 15 to Date When Ripe

Period	Location										
	Garden City	Tribune	Meade	Colby	Columbus	Dodge City	Hays	Kingman	Hutchinson	Manhattan	Thayer
Before heading	3.66[1] / 69 46[2]	3.67 / 68 45	4.46 / 70 45	2.29 / 69 43	1.53 / 72 52	2.61 / 66 48	2.43 / 71 48	2.77 / 73 50	2.60 / 72 50	1.64 / 71 47	2.34 / 73 51
During heading	1.96 / 78 56	3.42 / 76 53	1.48 / 80 53	2.34 / 79 53	0.76 / 76 55	1.92 / 79 58	2.21 / 80 59	1.08 / 84 59	1.65 / 81 58	2.06 / 80 57	1.34 / 80 56
After heading	8.18 / 82 58	2.92 / 82 55	4.14 / 83 55	5.04 / 81 55	6.48 / 82 61	5.58 / 80 59	6.29 / 84 59	5.22 / 85 60	5.99 / 83 62	4.20 / 81 61	6.42 / 81 62
Thiamin (mg./lb.)	1.99	1.97	1.97	1.89	1.84	1.77	1.71	1.70	1.64	1.61	1.46

[1] Precipitation (in inches) is given above the line. [2] Maximum and minimum temperatures (in °F.) are given below the line.

TABLE 2

Precipitation and Temperature for 1942, in Three Periods From April 15 to Date When Ripe

Period	Location										
	Garden City	Tribune	Meade	Colby	Columbus	Dodge City	Hays	Kingman	Hutchinson	Manhattan	Thayer
Before heading	4.03[1] / 69 44[2]	1.72 / 71 41	4.26 / 73 43	1.59 / 68 41	5.19 / 73 52	2.28 / 70 46	4.17 / 71 46	5.88 / 75 50	4.50 / 73 50	4.22 / 72 50	4.55 / 73 53
During heading	0.18 / 88 59	0.00 / 88 54	0.93 / 87 54	1.95 / 86 56	0.21 / 85 62	0.22 / 76 49	4.78 / 88 59	0.48 / 82 59	0.06 / 78 53	2.21 / 71 49	5.41 / 88 68
After heading	3.32 / 82 58	6.27 / 80 55	4.98 / 86 59	2.24 / 79 54	7.84 / 80 62	2.98 / 83 61	6.75 / 83 60	6.50 / 87 63	10.92 / 85 63	12.36 / 81 63	8.26 / 81 63
Thiamin (mg./lb.)	2.36	1.96	2.29	1.87	1.71	2.67	2.46	1.52	2.11	1.82	1.70

[1] Precipitation (in inches) is given above the line. [2] Maximum and minimum temperatures (in °F.) are given below the line.

TABLE 3

Chemical Analysis of 1941 Crop Samples [1]

Location	Test weight	Wheat			Flour yield (patent)	Flour		
		Protein	Ash	Thiamin		Protein	Ash	Thiamin
	lb.	pct.	pct.	mg./lb.	pct.	pct.	pct.	mg./lb.
Garden City	61.4	14.6	1.74	1.99	92.3	13.9	.42	.46
Tribune	58.6	13.0	1.55	1.97	92.7	12.0	.42	.41
Meade	54.1	14.6	1.97	1.97	91.8	13.7	.46	.46
Colby	57.9	13.1	1.54	1.89	92.9	12.1	.42	.38
Columbus	57.6	10.7	1.58	1.84	97.6	10.1	.41	.63
Dodge City	59.2	13.4	1.72	1.77	90.2	12.5	.43	.46
Hays	60.6	14.0	1.57	1.71	91.3	13.2	.42	.45
Kingman	57.3	13.5	1.79	1.70	92.0	12.6	.44	.50
Hutchinson	57.5	12.6	1.58	1.64	92.2	11.5	.42	.43
Manhattan	57.3	13.4	1.64	1.61	94.6	12.1	.41	.36
Thayer	58.8	11.8	1.64	1.46	92.4	10.8	.41	.50
Average	58.2	13.2	1.66	1.77	92.6	12.3	.43	.45

Variety

	Turkey	Tenmarq	Blackhull	Nebred	Chiefkan	Clarkan	Kawvale
Thiamin (mg./lb.)	1.97[2]	1.86	1.80	1.79	1.59	1.51	1.38
	0.39[3]	0.44	0.46	0.44	0.44	0.67	0.42

[1] Protein, ash, and thiamin contents expressed on 15 per cent moisture basis. [2] Wheat values given above the line. [3] Flour values given below the line.

TABLE 4

Chemical Analysis of 1942 Crop Samples [1]

Location	Test weight	Wheat			Flour yield (patent)	Flour		
		Protein	Ash	Thiamin		Protein	Ash	Thiamin
	lb.	pct.	pct.	mg./lb.	pct.	pct.	pct.	mg./lb.
Garden City	58.7	18.7	2.15	2.36	87.6	17.8	.45	.48
Tribune	59.8	16.2	1.78	1.96	89.7	14.9	.42	.56
Meade	58.5	16.1	2.00	2.29	90.9	15.0	.43	.55
Colby	58.5	14.4	1.82	1.87	86.2	13.4	.41	.35
Columbus	55.9	10.8	1.77	1.71	96.6	9.7	.42	.63
Dodge City	57.6	16.1	2.00	2.67	88.0	15.1	.45	.37
Hays	57.6	16.6	1.89	2.46	88.1	15.9	.42	.52
Kingman	57.0	14.7	1.70	1.52	87.7	12.7	.43	.29
Hutchinson	59.5	13.7	1.56	2.11	92.4	12.4	.41	.53
Manhattan	55.7	13.8	1.86	1.82	98.9	12.5	.41	.59
Thayer	57.9	12.6	1.60	1.70	95.0	11.5	.42	.62
Average	57.8	14.8	1.83	2.03	91.3	13.7	.42	.50

Variety

	Turkey	Tenmarq	Blackhull	Nebred	Chiefkan	Clarkan	Kawvale
Thiamin (mg./lb.)	2.05[2]	2.29	2.07	2.23	1.82	1.53	1.44
	0.42[3]	0.49	0.55	0.55	0.41	0.81	0.53

[1] Protein, ash, and thiamin contents expressed on 15 per cent moisture basis. [2] Wheat value given above the line. [3] Flour values given below the line.

content than were the wheats from the other stations. There was no demonstrable effect of location or variety on the thiamin content of the flours from the 1941 wheats.

Both location and variety also influenced the thiamin content of the 1942 wheat. The thiamin content of Tenmarq was significantly higher than that of Blackhull, Turkey, and Chiefkan but was not significantly greater in thiamin content than Nebred. The wheat from Dodge City was significantly higher in thiamin content than the wheats from all stations with the exception of that from Hays. For the 1942 flours both the location and varieties influenced the thiamin content.

Seasonal variation was shown to influence the thiamin content of wheat because the thiamin content of the 1942 wheat crop was 15 per cent higher than that of the 1941 crop; also, the ash and protein contents were considerably higher than those of 1941.

THIAMIN AS RELATED TO PROTEIN AND ASH CONTENT

Statistically, no significant relationship was found to exist between thiamin and wheat ash or wheat protein for either the 1941 or 1942 crops. These results are in agreement with those of Hoffman, Schweitzer, and Dalby (1940) and Nordgren and Andrews (1941), who found no relation between thiamin and ash in winter wheats. Very definite trends were noted, however, when the protein and ash contents were plotted against thiamin content, but in nearly every case there appeared one or two locations which were very much out-of-line and these probably caused the nonsignificant correlation coefficients. No explanation can be offered at this time for these locations which failed to fit the trend.

THIAMIN AS RELATED TO RAINFALL AND TEMPERATURE

Swanson (1941) suggested that weather conditions during the heading and blossoming stages in the life of the wheat plant greatly influenced the composition of the wheat berry. In the present study a significant relationship was found to exist between the thiamin content and amount of precipitation and minimum temperature during the week following the blossoming of the 1941 wheat crop (Fig. 2). The locations, Thayer and Columbus, were omitted because they represented strictly soft wheat areas. As the rainfall increased during this interval, wheat higher in thiamin content resulted, and a correlation coefficient of 0.73 was obtained (P = 3 per cent). For the same period the average minimum temperature and thiamin content were negatively related, and a correlation coefficient of —0.67 was obtained (P = 5 per cent). This relationship indicates that during the period following blossoming, cool weather with increasing rainfall resulted in greater amounts of thiamin in the wheat berry.

For the same interval during the 1942 growing season no significant relationship was found between either the precipitation or temperature, although trends similar to the previous year were noted. It is thought that the scarcity of rain preceding and immediately following the heading period may be a possible explanation for the failure to find a similar relationship for 1942.
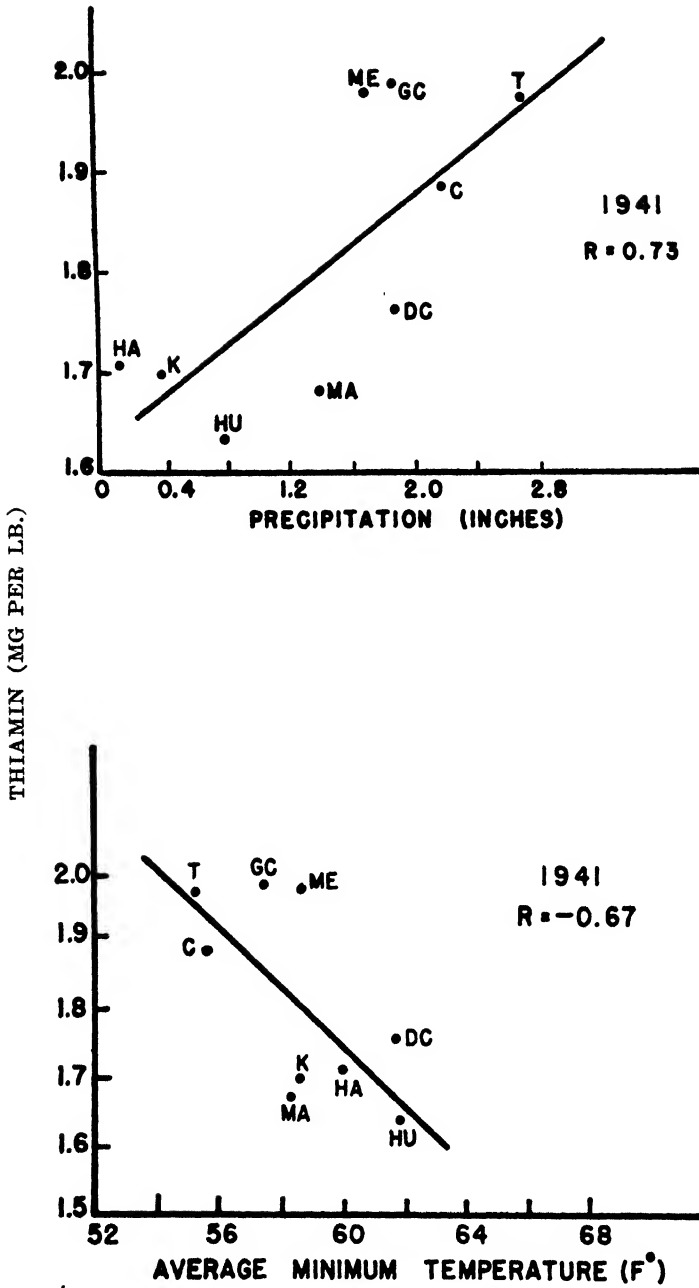
FIG. 2. Effect of precipitation and temperature upon thiamin content of wheat.

No correlation was found between the thiamin content of wheat and total precipitation (April 15 to harvest), although a nonsignificant negative correlation was noted between thiamin content of wheat and the amount of precipitation which occurred between heading and harvest for both 1941 and 1942, indicating that the thiamin content of wheat may be negatively related with conditions favoring starch formation. It is known that plentiful rains during the ripening period favor and accelerate the rate of starch synthesis. Further supporting evidence for this supposition is shown (Fig. 3), which gives the relationship between thiamin content
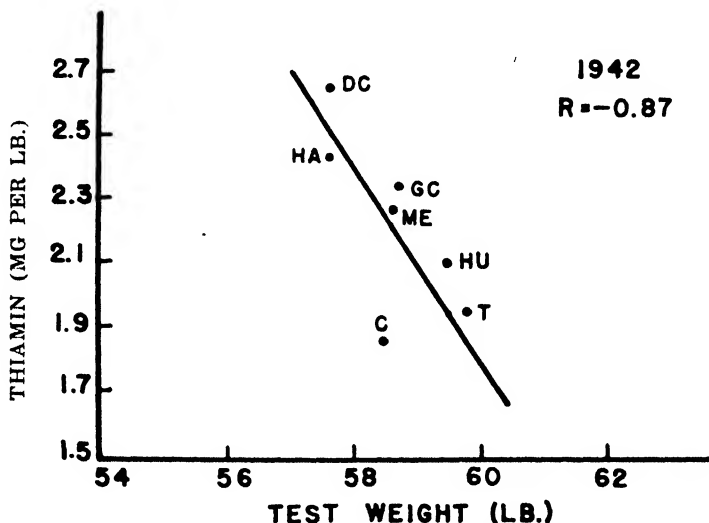
FIG. 3. Relationship between test weight and thiamin content of wheat.

and test weight of the wheat for 1942. A highly significant negative correlation coefficient of —0.87 was obtained, indicating that the thiamin content is higher in low-test-weight wheat than in high-test-weight wheat. The stations, Manhattan and Kingman, are omitted from this figure because the wheat was of very low test weight owing to severe weathering, as was seen by texture analysis. This relationship between thiamin content and test weight of wheat was not significant for 1941, but this might be explained by the greater variation in the 1941 test weights than in the following year owing to the much greater precipitation in 1941.

THIAMIN IN WHEAT AS RELATED TO THIAMIN IN FLOUR

At one time it was assumed that wheat with a high thiamin content would yield a flour that was higher in thiamin content than a flour milled from a wheat low in thiamin content. Statistically, the results obtained in this investigation show no relationship between the thiamin content of wheat and flour for either 1941 or 1942. The failure of wheats of high thiamin content to result in flours higher in thiamin content than flours milled from wheats of lower thiamin content is undoubtedly due to the fact that the greater portion of thiamin is located in the scutellum tissues.

Hinton (1942) has presented an extensive piece of research work indicating that the thiamin content of the scutellum (tissue immediately surrounding the germ) may be up to ten times that of the embryo. From the present knowledge of normal milling practices it is believed that the scutellum tissue is not included in patent grades of flour.

TABLE 5

Comparison of Thiamin Contents of Flours Milled on 65-Barrel Mill and Experimental Mill [1]

Variety	Wheat thiamin	Mill and grade	Yield[2]	Patent	Clear	Flour		
						Thiamin	Protein	Ash
	mg./lb.		*pct.*	*pct.*	*pct.*	*mg./lb.*	*pct.*	*pct.*
Tenmarq......	1.48	65 bbl. straight	71.1	......	......	.62	11.4	.44
Tenmarq......	......	Expt. straight	71.8	......	......	.66	11.9	.46
Tenmarq......	......	Expt. patent 96%	72.5	69.6	2.9	.48	11.4	.42
Pawnee........	1.14	65 bbl. straight	75.2	......	......	.56	11.9	.38
Pawnee........	......	Expt. straight	75.5	......	......	.58	12.2	.40
Pawnee........	......	Expt. patent 100%	73.9	73.9	0.0	.58[3]	12.0	.40
Turkey.........	1.04	65 bbl. straight	73.0	......	......	.53	11.6	.42
Turkey.........	......	Expt. straight	74.7	......	......	.55	11.9	.46
Turkey.........	......	Expt. patent 95%	72.6	69.0	3.6	.40	11.6	.42
Chiefkan.....	1.11	65 bbl. straight	69.6	......	......	.50	12.4	.51
Chiefkan.....	......	Expt. straight	73.1	......	......	.55	12.8	.52
Chiefkan.....	......	Expt. patent 88%	72.6	63.8	8.8	.41	12.6	.48
Blackhull....	1.20	65 bbl. straight	69.3	......	......	.50	12.2	.42
Blackhull....	......	Expt. straight	69.9	......	......	.54	12.6	.45
Blackhull...	......	Expt. patent 94%	69.8	65.6	4.2	.39	12.2	.42

[1] Thiamin, protein, and ash contents on 15 per cent moisture basis. [2] Dry-moisture basis.
[3] Same as straight flour.

The flours used in this investigation, as previously mentioned, were patent flours; the thiamin contents of flours milled on the Kansas State College 65-bbl. mill and the Allis-Chalmers experimental mill are shown (Table 5). The figures show that the thiamin contents of the flours milled on the large mill and the experimental mill are, for all practical purposes, identical and that any slight variability is well within the experimental error. It is considered that the patent flours used in this investigation represent reasonably well the type of flours which would be obtained from similar wheat if a commercial flour of the designated ash content were to be milled from such wheat.

SUMMARY

A study· was made with five winter wheat and two soft wheat varieties grown in Kansas to ascertain the effect of weather, variety, and location upon the thiamin cóntent of the wheat. In addition, the thiamin content was determined on the flour for each respective wheat, and the relationship between thiamin content of the wheat and of the flours was investigated.

Seasonal variation influenced the thiamin content of wheat. The thiamin content of the 1942 wheat crop was 15 per cent higher than that of the 1941 crop.

Thiamin content of wheat was influenced by both variety and location. The differences owing to variety and location are significantly different as shown by an analysis of variance.

No significant relationship was found between the thiamin and wheat ash or wheat protein although a positive trend was noted.

The thiamin content of wheat appeared to be negatively related with conditions favoring starch synthesis.

There appeared to be some factors which were not taken into consideration in this study and which may have prohibited the correlation of wheat ash or wheat protein with thiamin content; or else there was an excessive variation within the samples used.

Conditions favoring high percentage protein and ash appeared to favor high thiamin content.

No correlation was found between the thiamin content of wheat and flour milled from the wheat; therefore, wheat of high thiamin content does not necessarily mean that the flour milled from this wheat will be higher than flour milled from a wheat of low thiamin content.

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EFFECT OF CRUSHED ICE REFRIGERATION ON CONSERVATION OF VITAMIN C CONTENT AND ON RETENTION OF WEIGHT OF OHIO-GROWN VEGETABLES

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The rate of loss of vitamin C in vegetables varies with the conditions of holding, with different vegetables, and with different varieties of the same vegetable. The use of ice in holding some vegetables during transportation from distant markets is a rather common practice, but its use during transportation of vegetables from the local production areas to the local market and during holding in the market is very limited. In many cases, the garden-fresh appearance of locally grown vegetables has been lost by the time they are displayed for sale and therefore compare unfavorably with some which are shipped in from other areas. This study was undertaken in the interest of helping the local producer of vegetables to market his produce to advantage, to help the merchant to prevent waste, and to supply to consumers vegetables of fresh appearance and high nutritive value. It was thought that such a study would contribute information as to the kinds of vegetables that need to be packed in crushed ice during transportation and holding, and the length of time that certain vegetables may be held and still retain their fresh appearance and high nutritive value.

The loss of moisture in vegetables transported uniced and displayed in an open display case at room temperature without the use of ice becomes an economic factor when vegetables are sold by weight. The study of the rate of loss with certain vegetables and under certain conditions of storage would contribute some practical information as to the economic value of the use of crushed ice during transportation and holding.

REVIEW OF LITERATURE

The effect of storage at different temperatures on the ascorbic acid content of a number of vegetables was reported in a series of studies made by Tressler, Mack, and King (1936a, b); Tressler, Mack, Jenkins, and King (1937); Gould, Tressler, and King (1936); Mack, Tressler, and King (1936); Mack, Tapley, and King (1939); and Wheeler, Tressler, and King (1939). All vegetables studied, with the exception of rhubarb and tomatoes, lost ascorbic acid rapidly at room temperature in summer. In gen-

eral, the loss in all vegetables was less at refrigerator temperatures than at room temperature. The rates of loss at the different temperatures varied with the vegetables studied. Snap beans, lettuce, and kale lost vitamin C at a greater rate at 1 to 3°C.(33.8 to 37.4°F.) than did spinach, peas, lima beans, broccoli, cabbage, cauliflower, endive, cantaloup, parsnips, and kohlrabi at the same temperature. Cabbage lost only one-fifth of its ascorbic acid after storage for 19 days at room temperature, while spinach lost approximately one-half after three days at room temperature. Zepplin and Elvehjem (1944) reported an investigation on the ascorbic acid retention in Swiss chard, lettuce, spinach, broccoli, and green beans during various storage conditions. All these vegetables, except green beans, lost vitamin C quite rapidly at room temperatures of 20 to 23°C.(68 to 73.4°F.). Storage in refrigerators reduced the losses in these vegetables. Broccoli, Swiss chard, and lettuce retained most of their vitamin C when stored in crushed ice for three days. Gordon, Griswold, and Porter (1945) found that storage in snow ice favored the retention of vitamin C and the appearance and quality of leaf lettuce, green beans, and spinach.

EXPERIMENTAL PROCEDURE

The nutritive value of the vegetables studied was judged by the per cent retention of vitamin C, a criterion suggested by Fenton (1940). Weight changes were determined by use of the laboratory balance. All vegetables studied were grown on the Ohio State University Farm under the direction of the Horticulture Department, harvested, and brought to the laboratory at once. The weight and vitamin C content were determined and these values served as initial values on which all calculations of changes were based. Preliminary tests showed that the vitamin C content of samples placed in the extractant in the field was practically identical with that of those harvested and brought to the laboratory and then placed in the extractant. The period from time of harvesting to time of extraction was never more than 30 minutes.

The storage conditions under which the vegetables were held were as follows: exposed to air at room temperature, approximately 26.7°C. (80°F.); on top of crushed ice in an open display case; buried in ice in a drained metal tray; in refrigeration at approximately 10°C.(50°F.). These vegetables were held under these conditions as long as they were considered to be of marketable quality. The time of holding varied with the vegetable and the condition of storage.

The vitamin C contents of beets and asparagus were determined by the method of Roe and Oesterling (1944). All other vegetables were tested by the method of Morell (1941). Relative losses could be computed from the results obtained by the Morell method and many more samples could be analyzed in a shorter time by the latter method. Each time a vegetable lot was tested for vitamin C content, two different samples were chosen and analyzed separately. A mixture obtained by combining portions of the extractants in which the two samples were blended was also analyzed. This check method showed that variations of more than one per cent in the two samples could not be attributed to poor analytical procedure. Sampling methods varied with different vegetables. In general, however, the average

variation in vitamin C content of the two samples from the same lot was less than five per cent.

RESULTS AND DISCUSSION

Effect of Four Different Storage Conditions: Since the vitamin C retention tests for asparagus, peas, cabbage, Swiss chard, and lima beans covered similar storage conditions, the results will be presented in a combined form. For each of these vegetables vitamin C determinations were made when freshly harvested and at intervals after storage of from one to 10 days. Each vegetable was harvested several times and experiments were repeated.

In general, samples contained all edible portions of the vegetable being tested. Peas and lima beans were stored unshelled. Swiss chard samples were taken from the leaves of several plants. Asparagus samples contained several stalks. For cabbage, different samples from the same head were tested at various times.

The vitamin C data were collected into 20 tables, one for each of the five vegetables and four storage conditions. To evaluate the data, each table was examined for significant trends in vitamin C loss during storage and then the trends in different tables were compared. The discussion below indicates how this was done.

Three heads of cabbage, harvested at different times, were sampled for vitamin C content when freshly harvested and after being held at room temperature for from one to four days. The vitamin C content is expressed in milligrams per 100 grams of fresh weight (Table 1); each value was obtained by analyzing two radial slices from the same head of cabbage. For the 13 determinations the per cent differences between the duplicate samples ranged from 0 to 10 per cent, with a mean value of 3.9 per cent. This would suggest that the apparent vitamin C increase at the end of the first day might be due to sample variation, while the vitamin C losses after three and four days cannot be attributed entirely to this cause.

TABLE 1

Vitamin C Retention [1] of Cabbage Held at Room Temperature

Head No.	Number of days stored at 26.7°C. (80°F.)				
	0	1	2	3	4
1...	47.2	50.1	......	45.0	40.8
2...	73.6	73.9	......	64.9	66.7
3...	52.8	54.2	49.5	50.3	49.8
Average per cent retention..........	100.0	103.0	94.0	93.0	90.0

[1] Given in milligrams per 100 grams.

Vitamin C retention data for each vegetable under each storage condition were expressed as percentages of the corresponding freshly harvested value and these percentages were averaged. This simplified comparisons among the data. The average per cent retentions were smoothed in order to eliminate small variations which might obscure trends in vitamin C loss. The smoothing was accomplished by fitting exponential curves by the method of least squares. For the data of Table 1, the smoothing process gave per cent retentions of 98, 96, 93, and 90, respectively, for the first four days of storage.

Graphs of the curves mentioned above are shown (Fig. 1). The 100-per cent level on these graphs is equal to the following values in milligrams per 100 grams: cabbage, 66.1; peas, 39.0; chard, 35.6; lima beans, 34.7; and asparagus, 29.7. The graphs clearly show that the vitamin C retention varied greatly among the different vegetables. Cabbage retained most of its vitamin C under all four methods of storage, while chard lost considerable vitamin C in each case. It is also indicated that each individual vegetable retained its vitamin C best when packed in ice and suffered the greatest loss when exposed to air at room temperature.
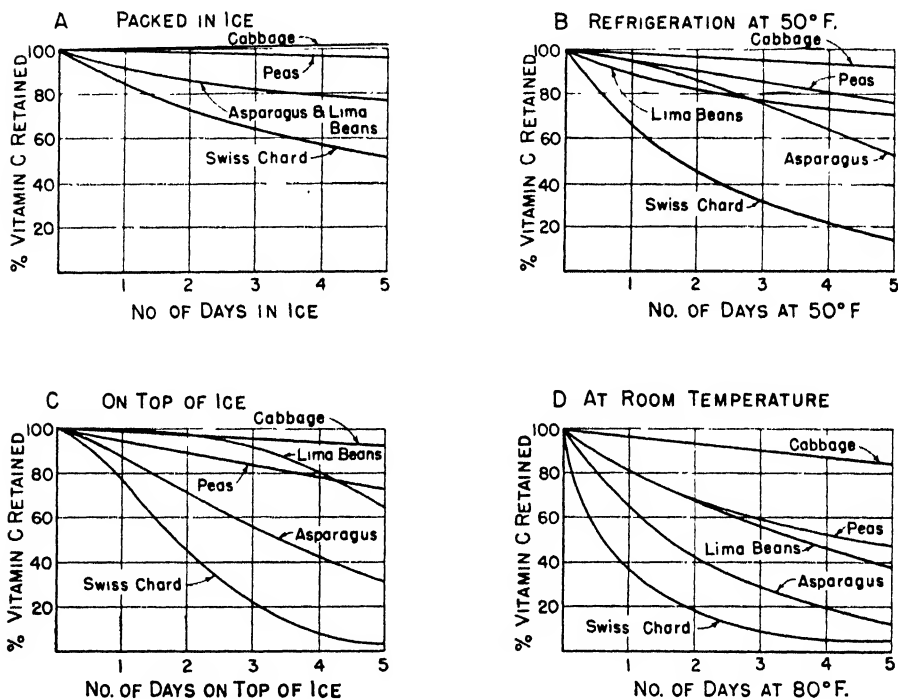
FIG. 1. Vitamin C retention as related to different vegetables, storage conditions, and length of storage period.

It was found that broccoli would not remain in a marketable condition for more than two days either at room temperature or when displayed on top of ice, although it would keep much longer under refrigeration. This suggested a study of vitamin C retention for a two-day period, making sufficient tests so that differences in vitamin C retention for different storage methods could be tested statistically for significance. Broccoli was held under the four storage conditions already mentioned except that the average refrigeration temperature was 5.6°C.(42°F.) instead of 10°C.(50°F.).

Samples, taken from what seemed to be the edible portion of a head, included the flowers, small leaves, and about three inches of stalk. For one head the vitamin C content, in milligrams per 100 grams, was found to be as follows: in the leaves, 180; in the flowers, 142; and in the stalk,

98. Among the different heads, apparently of the same maturity, the tested vitamin C content was found to vary from 83 to 174 milligrams.

In an effort to eliminate the effect of the great variation in vitamin C content of fresh samples the general practice in testing was to divide a freshly harvested head lengthwise. One half was tested immediately and the other half was tested after storage for one or two days. The vitamin C content of each stored sample was then expressed as a percentage of the vitamin C content of the corresponding fresh sample.

An average of eight separate tests was made for each method of holding. The average vitamin C content of freshly harvested broccoli was 132 milligrams per 100 grams fresh weight; the average per cent retention is shown (Table 2).

TABLE 2

Vitamin C Retained in Broccoli

Method of storage	Number of days stored	
	1	2
	pct.	*pct.*
Packed in ice..	101	103
Refrigerated at 5.6°C.(42°F.)................................	93	92
On top of ice..	89	91
Room temperature of 26.7°C.(80°F.).....................	70	56

The usual statistical methods, e.g., Snedecor (1940), were used to test for significant differences between two means. It was found that for each day the vitamin C content of broccoli packed in ice was significantly greater than for the other methods of storage. The difference between refrigeration and storage on ice was not significant but these methods were significantly better than holding at room temperature. The differences between the first and second days were not significant except for storage at room temperature. The apparent increases when the broccoli was packed in ice could not be regarded as significant, but the vitamin C loss was significant for each other method of storage.

Effect of Different Storage Temperatures: Storage temperature seemed to be the most important factor in the vitamin C retention for a particular vegetable. A short experiment, designed to test this observation, was carried out with shelled lima beans which were packed in crushed ice and were held under refrigeration both at 4.4 and 10°C.(40 and 50°F.). The results, smoothed by the method used for cabbage, show the per cent of the original vitamin C retained, at the end of 24-hour periods, for five days (Table 3). They indicate that vitamin C loss in shelled lima beans is greater at higher refrigeration temperatures.

Comparison of Vitamin C Retention and Weight Changes for 14 Vegetables: It has been noted that for one method of storage the vitamin C retention of two different vegetables may vary widely. To obtain more information about this variation, additional experiments were carried out using beets, carrots, celery, green peppers, kale, radishes, New Zealand spinach, and tomatoes. Each of these vegetables was tested for vitamin C content when freshly harvested, after one and two days' storage in ice,

and after one and two days at room temperature. These results, along with the corresponding data for the vegetables studied earlier, are presented (Table 4).

For each of the 14 vegetables studied, samples were weighed when freshly harvested and then reweighed after various intervals of storage. The resulting weight-change data showed that, in general, a rapid loss of weight occurred at room temperature, the loss was less rapid under refrigeration, and there was no weight loss for vegetables packed in ice. Observed weight changes for each vegetable after two days in ice and after two days in air are also shown (Table 4).

TABLE 3

Retention of Vitamin C in Shelled Lima Beans

Method of storage	Number of days stored				
	1	2	3	4	5
	pct.	*pct.*	*pct.*	*pct.*	*pct.*
Packed in ice........................	92	84	78	71	65
Refrigerated at 4.4°C. (40°F.).......	86	75	68	63	61
Refrigerated at 10°C. (50°F.)........	84	70	57	45	35

TABLE 4

Vitamin C Content and Weight Changes of Vegetables Packed in Ice for Two Days and of Vegetables Held at Room Temperature for Two Days

Vegetables	Vitamin C (milligrams per 100 grams)					Weight (per cent of) original	
	Freshly harvested	After 1 day in air	After 1 day in ice	After 2 days in air	After 2 days in ice	After 2 days in air	After 2 days in ice
Asparagus....................	30	20	28	13	26	80	104
Beets............................	4	3	3	3	3	89	102
Broccoli........................	132	93	134	74	136	58	105
Cabbage........................	66	64	67	62	67	93	102
Carrots.........................	9	10	11	5	8	83	102
Celery...........................	20	15	19	11	20	71	102
Green peppers..............	151	140	129	128	134	95	100
Kale..............................	143	121	150	91	128	70	108
Lima beans (in pod)..	35	28	32	23	30	94	100
New Zealand spinach..	41	28	38	20	36	69	122
Peas (in pod)..............	39	32	39	26	38	86	100
Radishes........................	30	25	29	21	28	80	103
Swiss chard..................	36	13	31	6	27	65	115
Tomatoes......................	26	26	26	23	25	99	100

This table clearly reveals the effectiveness of ice in retaining the original weight and the vitamin C content of vegetables. When packed in ice for two days most vegetables actually gained weight, the average increase being about four per cent. The weight losses of vegetables held at room temperature for two days varied considerably but averaged about 20 per cent. Swiss chard and beets were the only vegetables to lose more than 15 per cent of their original vitamin C content when packed in ice for two days. On the other hand, only cabbage, tomatoes, and peppers

retained more than 85 per cent of their original vitamin C content after being held at room temperature for two days.

SUMMARY

Vegetables were studied to determine the rates of loss of vitamin C when the vegetables were held at room temperature, on top of crushed ice, buried in crushed ice, and in refrigerators. The vegetables were held under each of these conditions for periods of from two to 10 days. Weight changes were determined for these vegetables after storage for two days at room temperature and storage for two days in crushed ice.

The rate of loss of vitamin C during storage varied with the vegetables studied and the storage conditions. Cabbage, tomatoes, and green peppers retained 94, 88, and 85 per cent, respectively, of their original vitamin C content when stored in air at room temperature for 48 hours. All other vegetables studied showed a much lower per cent retention under this storage condition.

In every case the vegetables stored in crushed ice retained a greater percentage of their vitamin C than those stored under any of the other three storage conditions.

Vegetables stored in crushed ice showed an average increase in weight of about four per cent in two days. Leafy vegetables, such as spinach, chard, and kale, showed the greatest increases.

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DETERMINATION OF IRON IN FOODS AND FOOD PRODUCTS

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• The iron content of foods and food products is important for several reasons. It is significant from the viewpoint of nutrition as well as for the possible effect on stability. Whatever the reasons, accurate and rapid methods for its determination are essential. The well-established thiocyanate method for iron is gradually being replaced by procedures which employ reagents, such as alpha, alpha'-dipyridyl [Cereal Laboratory Methods (1941), Jackson (1938), and Moss and Mellon (1942)] and 1,10-phenanthroline [Benne and Snyder (1944), Cowling and Benne (1942), Fortune and Mellon (1938), and Pyenson and Tracy (1945)] that yield stable-colored iron complexes not readily affected by variations in the hydrogen-ion concentration or by the presence of other ions. The work of Woods and Mellon (1941) has established the reason for this trend and clearly indicates the inferiority of the thiocyanate reagent. While many of the analyses made in the past using thiocyanate reagent were accurate, some of the data reported indicate either a marked and unusual variation in the iron content of some products or errors in some of the analyses.

The iron contents of calf liver and beef liver have been reported by Elvehjem and Hart (1926) to be 54 and 83 p.p.m., respectively, and by McCance and Widdowson (1940) to be 240 and 240 p.p.m., respectively. The cause of such variations may be in the age of the animal, the condition of the animal at the time of slaughter, or the methods used to determine the iron.

Another case of wide difference is that of the reported iron content of milk. Data in the literature vary over a range of 0.2 to 58 p.p.m. [Johnson (1944) and Stugart (1931)]. Rogers and associates (1935), Sherman (1941), and Reis and Chakmakjian (1932) place the iron content of milk between 1 and 4.5 p.p.m., while Stugart (1931) and Johnson (1944) found 0.1 to 0.7 p.p.m. Recent analyses made by the authors are in agreement with the latter figures (Table 1).

The work reported in this paper includes an investigation of methods and their application to liquid and dried milk, fats and oils, and meat and meat products.

EXPERIMENTAL PROCEDURE

The usual procedure for the preparation of samples is to consume the organic matter either by ashing the sample in the dry form, according to Cereal Laboratory Methods (1941), or by acid digestion, according to Jackson (1938), Reis and Chakmakjian (1932), Stugart (1931), and Thompson (1944). The latter has been used extensively, but in the experience of the authors this method requires more time and attention by the analyst than does dry ashing. Dry ashing of the sample can be

accomplished by heating in an electrically heated muffle furnace at 500 to 550°C. (932 to 1022°F.) for four to six hours. No harm is done if the sample is heated for 16 hours (overnight). It is claimed that dry ashing gives low results owing to the presence of phosphates, but the authors did not encounter any such trouble when the temperature was maintained at 500 to 550°C. during ashing. In fact, just the opposite was observed in the case of whole milk (Table 1). In the case of samples of whole milk

TABLE 1

Comparison of Wet and Dry Ashing Procedures

Sample		Dry ashing				Wet ashing	
		1,10-phenanthroline (iron in p.p.m.)			Thiocyanate (iron in p.p.m.)	1,10-phenanthroline (iron in p.p.m.)	Thiocyanate (iron in p.p.m.)
Liquid whole	1	0.6,	0.5,	0.5	....	0.4	0.3
milk	2	0.5,	0.5,	....	....	0.3	0.3
	3	0.7,	0.6,	....	....	0.4	0.3
	4	0.5,	....	....	....	0.3	0.3
	5	0.5,	0.5,	0.7	....	0.3	0.4
	6	0.6,	....	....	0.5	0.3	0.3
	7	0.7,	....	....	0.6	0.2	0.2
	8	0.7,	....	....	0.6	0.4	0.3
Dried whole	1	5.0,	5.5,	5.0	....	....	5.2
milk	2	4.5,	5.0,	....	....	....	4.5
	3	5.0,	....	....	....	....	....
	4	7.5,	....	....	....	....	....
	5	3.0,	....	....	....	....	....
	6	6.5,	....	....	....	....	....
Special mixtures							
Blank[1] on mixture No. 1		0.5			0.5	0.4	0.4
Mixture No. 1 plus iron (2 p.p.m.)		2.6			2.3	2.4	2.2
Calculated iron content		2.5			2.5	2.4	2.4
Blank[2] on mixture No. 2		0.9			....	....	0.7
Mixture No. 2 plus iron (2 p.p.m.)		2.7			....	....	2.1
Calculated iron content		2.9			....	....	2.7

[1] Special mixture No. 1 taken for analysis contained 1.25 gm. urea, 3.75 gm. sugar, and 0.01 gm. calcium phosphate made to a total of 50 gm. with water.   [2] Same composition as above except 0.20 gm. calcium phosphate in place of 0.01 gm.

and special mixture No. 2, apparently a portion of the iron was converted during wet ashing to an inactive form which did not react with the color-producing reagents. Platinum and Vycor dishes were used for dry ashing with equally satisfactory results.

Other difficulties were experienced when using the wet ashing procedure, Thompson (1944). If the sample was insufficiently heated, the perchloric acid was not entirely expelled and an abnormally intense color was produced when the thiocyanate reagent was added. This, of course,

led to false iron values considerably above the true iron content. Excessive heating invariably led to low results. If carried nearly to dryness, the iron value obtained could be made to approach zero. The addition of sulfuric acid to maintain the original volume of the solution gave a slight improvement, but results still fell short of the true value. Special care had to be taken to wash down the necks of the digestion flasks with water

TABLE 2

Effect of Copper Ion, Reducing Agent, and pH

Sample	Copper added to sample [1]	Reducing reagent used [2]	pH of test solution	Iron
	p.p.m.			*p.p.m.*
A	....	HQ	3.6	66
A	....	HQ	4.4	66
A	....	HQ	4.9	66
A	....	HH	3.5	66
A	....	HH	4.9	66
A	3	HQ	3.5	64
A	3	HQ	4.4	64
A	3	HQ	4.9	64
A	3	HH	3.5	64
A	3	HH	4.9	64
B	....	HQ	1.4	10
B	....	HQ	4.4	27, 31
B	....	HH	1.5	9
B	....	HH	4.4	28
B	3	HQ	1.4	14
B	3	HQ	4.4	32
B	3	HH	1.4	12
B	3	HH	4.4	32
C	....	HQ	4.0	6.5
C	3	HQ	4.0	6.0
D	....	HQ	4.4	26
D	3	HQ	4.4	26
E	....	HQ	3.7	5.0
E	3	HQ	3.7	5.0
F	....	HQ	4.0	4.0
F	....	HQ	5.5	4.0

[1] Copper added as copper sulfate, copper content of original samples was approximately 1.5 p.p.m., making the total copper present approximately 4.5 p.p.m. [2] HQ = hydroquinone; HH = hydroxylamine hydrochloride.

during the two washing operations. Failure to do this permitted small amounts of nitric acid to remain, the result of which was the development of a deep red color when the thiocyanate was added. These difficulties and the great amount of time and attention required by wet ashing were the reasons for adoption by the authors of the dry-ashing technique. This has been used throughout except where otherwise specified.

In the investigation for determination of iron in milk and milk products, both alpha, alpha'-dipyridyl and 1,10-phenanthroline were used. Low

results were obtained with alpha, alpha'-dipyridyl, Cereal Laboratory Methods (1941), unless the effect of the phosphate ions was eliminated by adding sulfuric acid and phosphoric acid, as prescribed by Moss and Mellon (1942). The effect of phosphate ion on 1,10-phenanthroline can be eliminated simply by heating the ash with five ml. of dilute hydrochloric acid (1:1) to boiling when dissolving the ash. This is simpler than adding special reagents, so 1,10-phenanthroline was selected as the better of the two reagents for routine analysis.

TABLE 3

Effect of Buffer Solutions and Reduction of Hydrochloric Acid Content on pH of Final Test Solution

Proportion of hydrochloric acid used in test to that added in an analysis	pH of test solution using buffer solution		
	A	B	C
pct.			
0	4.3	4.7	6.4
20	4.0	4.5	5.4
40	3.6	4.3	5.0
60	2.0	4.1	4.6
80	1.5	3.9	4.4
100	1.2	3.5	4.0

Composition of Buffer Solutions A, B, and C

Buffer	Sodium acetate	Acetic acid	Water
	gm.	*ml.*	*ml.*
			Diluted
A[1]	16.6	24	to 200
B	20.0	10	80
C	20.0	....	80

[1] Pyenson and Tracy (1945).

The effects of (1) the presence of copper which is frequently found in dried whole milk, (2) the use of hydroquinone or hydroxylamine hydrochloride to reduce the iron to the ferrous state, and (3) the pH of the final test solution, on the determination of iron with 1,10-phenanthroline, were investigated in some detail. A solution of copper sulfate in water was added to several samples to provide an amount several times that normally found in dried whole milk. The results (Table 2) indicate that copper in the quantities indicated did not affect the results. Both a 2.5-per cent solution of hydroquinone and a 10-per cent solution of hydroxylamine hydrochloride using two ml. of each appeared to be satisfactory (Table 2).

The pH value of the test solution and means of regulating the pH were investigated. Cowling and Bene (1942) prescribe taking an aliquot of the sample and titrating it with a sodium acetate solution to a specified pH to determine how much buffer to add. This may be necessary for the most precise control over pH and for the most accurate results. However, in routine analysis it is considerably more convenient to be able to add a fixed amount of buffer solution. This, of course, would allow the pH of

the final test solution to vary within some limits determined by the amount and composition of the buffer, the amount of alkaline ash, and the amount of hydrochloric acid volatilized when heating the acid solution to dissolve the ash and to eliminate the phosphate ion interference. It has been shown by Fortune and Mellon (1938) that if the pH is kept within the limits of 2 to 9 there should be little or no variation in the final results. The pH measurements of the final test solutions, when fixed amounts of different buffers (five ml.) are added to tests containing 0 to 100 per cent of the five ml. of dilute hydrochloric acid (1:1), are given (Table 3). In view of the above results, Buffer A was considered unsatisfactory. The effects of low pH on analyses are shown (Table 2). Either Buffer B or C would be satisfactory (Table 3), but Buffer C was selected because it provided a greater margin of safety should the specified amount of acid be exceeded and because the excess sodium acetate had no effect on the analysis.

TABLE 4

Comparison of Wet and Dry Ashing Procedures on Canned Meats

Sample	Iron determined by	
	Wet ash-thiocyanate method	Dry ash-1,10-phenan-throline method
	p.p.m.	p.p.m.
Strained		
Beef	28	28
Veal	18	16
Lamb	20	23
Pork	14	17
Calf heart	38	36
Beef liver	72	77
Cubed		
Beef	35	35
Veal	10	12
Lamb	17	21
Pork	13	15
Calf heart	38	46
Beef liver	65	67

A comparison of analyses of meat by the dry ashing-1,10-phenanthroline method and by the wet ashing-thiocyanate method (Table 4) shows that results obtained by the latter were lower. This is in accord with the analyses of whole and dried milk as shown (Table 1).

The dependability of the dry ashing-1,10-phenanthroline method to account for a known amount of iron added to a sample was checked by analyzing some fats, oils, and meats with and without added iron. The results of these tests are tabulated (Table 5) and show that the iron added to the samples was accounted for in all cases.

Results on some fresh meats (fat trimmed away) are tabulated (Table 6). Series 1 and 2 were collected a week apart, eliminating the possibility of getting duplicate samples from the same animal. It is interesting to observe the constancy of the iron content of all specific cuts of beef, veal,

TABLE 5

Analyses for Iron in Fats, Oils, and Meats With and Without Added Iron

Sample	Iron found in original sample	Iron added to sample	Total iron in sample calculated	Iron found by analysis
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Beef	26.0	10	36.0	34
Lamb	23.0	10	33.0	34
Pork	13.0	10	23.0	24
Beef	27.0	10	37.0	38
Lamb	24.0	10	34.0	33
Pork	16.0	10	26.0	26
Cottonseed oil	0.5	10	10.5	10
Soybean	0.5	10	10.5	10
Peanut	0.5	10	10.5	10
Lard	1.0	10	11.0	11
Tallow	3.0	10	13.0	12

TABLE 6

Analyses of Meats

Sample	Loin		Round		Heart		Liver		
							1	2	
	1	2	1	2	1	2		End	Center
Beef	21	27	22	27	48	54	56	54	54
Veal	16	15	17	16	46	55	180	54	54
Lamb	22	22	27	24	50	46	50	103	94
Pork	9	7	10	16	42	44	342	210	215

(Header: Iron (p.p.m.) in)

TABLE 7

Analyses of Meat Products

Sample	Iron in sample	
	1	2
	p.p.m.	*p.p.m.*
Frankfurters	15.6	13.8
Cooked, canned pork	17.2	18.4
Salami	26.8	24.5
Bologna	13.5	25.9
Meat loaf	14.7	21.9
Liver sausage	176.0	......
Pork sausage	17.2	14.0

pork, and lamb, except in the case of liver. The cause of the wide variation in iron content of livers is outside the scope of this paper, but it does not lie in the method or sampling. The distribution of iron throughout the liver appears to be uniform since samples taken from the end and center differ by less than the probable experimental error.

Analyses of some meat products are given (Table 7).

The precision of the method as expressed by the standard deviation includes not only variables within the method but also those variables arising from nonuniformity within the sample. Several determinations were made on single samples of hamburger, dried whole milk, and strained lamb, and the standard deviations were calculated (Table 8). There is no explanation of the two high iron results for the hamburger sample except perhaps contamination from some outside source.

TABLE 8
Determination of Standard Deviation

	Iron found in		
Hamburger		Strained lamb	Dried whole milk
p. p. m.		*p. p. m.*	*p. p. m.*
	23.1	23.5	16.8
	23.8	23.7	16.2
	22.2	24.9	15.0
	33.8[1]	23.3	15.6
	22.2	24.1	15.6
	28.4[1]	23.7	14.8
	23.1	23.1	15.0
	22.8	23.1	15.0
	24.6	24.1	16.2
	22.4	22.9	16.2
	22.3	23.1	14.9
	24.4	23.9	......
Average	23.1	23.6	15.6
Standard deviation	±0.85	±0.55	±0.65

[1] Values omitted in calculating average and standard deviation.

The iron may be determined by visual comparison of the color developed in the test solution with those of standard iron solutions or by measuring the transmittance of the test solution at a selected wave length between 500 and 512 millimicrons and then reading the iron content from a concentration-transmittance-calibration graph. The latter procedure was employed in this study and is recommended since it is more accurate and the personal factors do not enter into the color reading. All spectrophotometric measurements were made with a Model 11 Coleman spectrophotometer at wave length 503 millimicrons with the PC-4 filter using 19 x 150 mm. round cuvettes. The calibration curve used in our analyses is shown (Fig. 1).

Reagents:

Hydrochloric acid solution (1 vol. HCl of sp. gr. 1.18, reagent quality and 1 vol. distilled water).

Hydrochloric acid solution, dilute (1 vol. HCl of sp. gr. 1.18, reagent quality and 19 vol. distilled water).
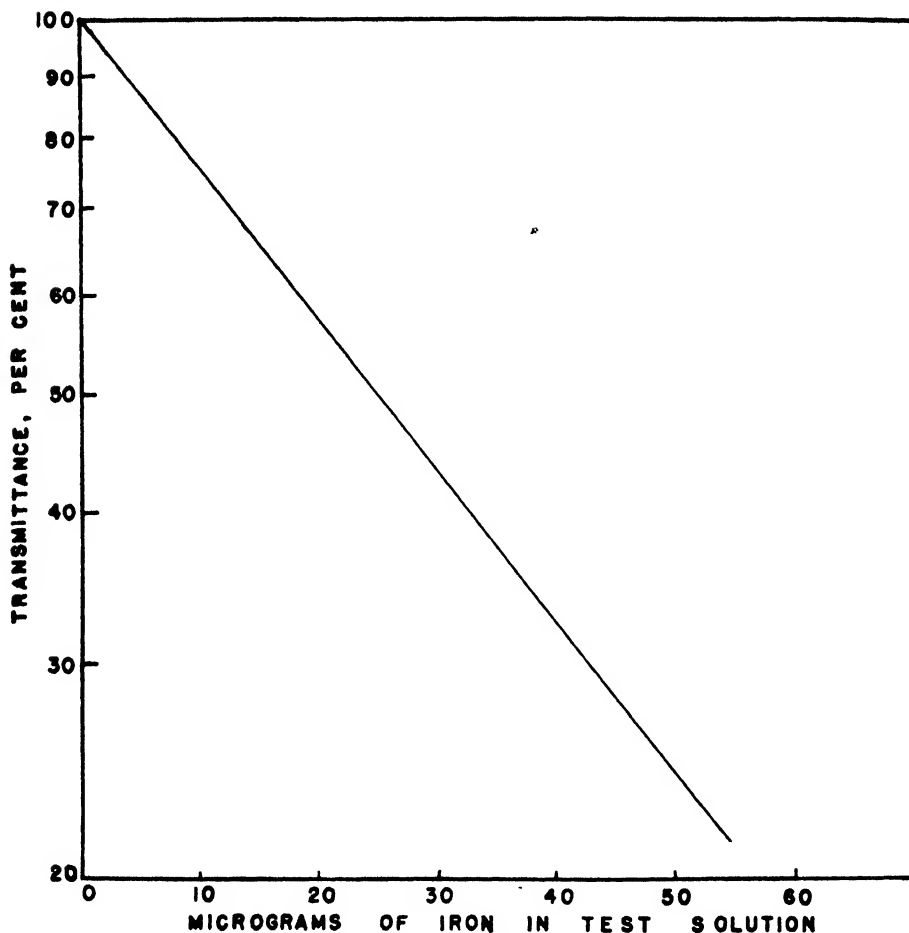
FIG. 1. Concentration-transmittanse-calibration graph for the determination of iron with 1,10-phenanthroline.

Hydroquinone solution, 2.5 gm. hydroquinone, 1 ml. of (1:1) HCl, and dilute to 100 ml. with distilled water.

1,10-phenanthroline solution, 0.1 per cent in distilled water.

Sodium acetate solution, 20 per cent by weight in distilled water.

Standard Iron Solutions: (a) Standard iron solution: Dissolve 0.1000 gm. pure iron wire in 10 ml. of 10 per cent sulfuric acid and 3 ml. nitric acid (sp. gr. 1.43) and dilute to 1 liter with distilled water in a volumetric

flask. (b) Diluted standard iron solution: Pipet 100 ml. of the standard iron solution into a 1-liter volumetric flask and dilute to mark with dilute HCl (1 vol. HCl and 19 vol. distilled water).

<div style="text-align:center">PROCEDURE</div>

Ash five grams of sample (more when it contains less than five p.p.m. of iron) in a platinum dish in a muffle furnace. Raise the temperature slowly until the sample is completely charred. Then raise the temperature to 500°C. and maintain at 500 to 550°C. until the sample is a greyish white. This usually requires four to six hours, but no harm is done if the sample is allowed to remain in the muffle overnight. Add five ml. hydrochloric acid (1:1) to the ash, cover the dish with a watch glass, and heat just to boiling. Transfer the sample to a 50-ml. volumetric flask using distilled water to wash the last traces from the dish. Dilute to the mark with distilled water, mix thoroughly, and pipet 10 ml. into a cuvette used for the transmittance measurements. When the iron content is too high to be read from the calibration graph, take a smaller aliquot and dilute to 10 ml. Add two ml. of hydroquinone, 5 ml. of 1,10-phenanthroline solution, and five ml. of the 20 per cent sodium acetate solution. These three reagents are added with volumetric pipets and the solution is mixed thoroughly after each addition.

A blank is run along with the sample to be sure that none of the reagents are contaminated and for a reference solution against which the transmittance of the sample is measured. The transmittance of the blank is usually about two per cent less than that of distilled water, but if it is five per cent less, new reagents are to be prepared.

The transmittance of all solutions is measured at 503 millimicrons with the instrument adjusted to read 100 per cent transmittance for the blank. The iron content of the test solution is read from the concentration-transmittance-calibration graph and the iron content of the sample calculated.

<div style="text-align:center">PREPARATION OF CONCENTRATION-TRANSMITTANCE-CALIBRATION GRAPH</div>

Pipet 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. of diluted standard iron solution (10 micrograms of iron per ml.) into a series of spectrophotometric cuvettes. Make up to 10 ml. with dilute hydrochloric acid solution (1:19) by adding the required amount from a measuring (Mohr type) pipet. Follow the above described procedure beginning with the addition of the two ml. of hydroquinone. Plot the per cent transmittance of the solutions against the micrograms of iron added.

<div style="text-align:center">SUMMARY</div>

A simple and accurate method is presented for the determination of iron in food products, such as milk, dried milk, meats, meat products, fats and oils. This method involves dry ashing the sample and determining the iron colorimetrically with 1,10-phenanthroline. Phosphates and copper in quantities normally present in these products do not interfere with the determination of iron. For routine analysis, dry ashing at 500 to 550°C. proved superior to wet ashing. Tests indicate that Vycor can be used in

place of platinum if the ash is not so alkaline that it attacks the Vycor. Analyses are given for milk, dried whole milk, meats, meat products, fats, and oils.

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EFFECT OF COMMERCIAL CURING, SMOKING, STORAGE, AND COOKING OPERATIONS UPON VITAMIN CONTENT OF PORK HAMS

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Although there have been numerous reports on the extent to which vitamins are retained in meats during different methods of home cooking, there have been few published investigations of the effect of commercial curing and smoking processes. Since a large percentage of the pork consumed in this country is either cured or cured and smoked, information regarding the retention of vitamins during these operations is essential for a complete understanding of the possible losses during marketing and preparation of pork for consumption.

Schweigert, McIntire, and Elvehjem (1943) have published data indicating that during the curing and smoking of a single ham the retentions of thiamin, riboflavin, and niacin were 73, 92, and 84 per cent, respectively. Unfortunately, these percentages of retention were calculated on a basis of the residual solids (i.e., free of fat and moisture) of companion hams, one being sampled before and the other after curing and smoking. Such a basis for calculation did not take into account the presence of the considerable quantity of curing salts which must have appeared in the residual solids of the cured and smoked ham but not in the fresh ham. Revised calculations and additional data obtained by these workers, Schweigert, McIntire, and Elvehjem (1944), indicated that the actual retentions during curing and smoking were 10 to 20 per cent higher than the earlier report, being 80 to 85 per cent for thiamin, 97 to 106 per cent for riboflavin, and 100 to 107 per cent for niacin.

The series of experiments here reported has been conducted in such a manner that the retention of vitamins during each operation commonly carried out in the commercial processing of hams can be determined as well as the over-all retention after a series of such operations. The processes that have been investigated are curing, smoking, storage of smoked hams prior to sale, storage of cured hams in freezers with subsequent smoking, and the preparation of cooked hams. In addition, the effects of roasting fresh hams and smoked hams has been investigated in order to compare the over-all effect of curing-smoking-roasting to that of roasting fresh ham. These studies were conducted in three series as described below.

EXPERIMENTAL PROCEDURE

Experiment 1: Eighteen pairs of companion hams, U. S. Good in quality, were obtained directly from the pork-processing factory, weighed, numbered, and divided into sets of right and left hams. Slices two inches in thickness were cut from the center of each of the left hams. Each slice was trimmed free of visible fat and bone, ground, frozen, and stored at

—29°C.(—20°F.) until needed for comparison with corresponding slices from the respective processed companion hams. The right hams were carried through the plant operations (discussed later) in the regular production line by skilled factory employees.

In accordance with general curing practice of the industry, all of the right hams were pumped with sweet-pickle brine,[1] packed in tierces with other hams, and stored under cover pickle [1] at 2°C.(35°F.) for two weeks. At the end of this period the hams were removed from cure, weighed, and treated as follows:

Two hams were taken immediately for analysis to show the effect of the curing operations.

Six hams were smoked with other hams undergoing the regular smoking treatment. This process involved the application of sufficient dry heat over a period of several hours to raise the internal temperature to 58°C.(137°F.) or over. After smoking, the hams were chilled for 24 hours in the smoked-ham cooler. Then two were taken for analysis to show the effects of curing and smoking. The others were stored for 10 days (two hams) and 30 days (two hams) at 2 to 3°C.(35 to 38°F.) before analysis. These conditions were selected as representing average and maximal storages for this type of ham under conditions which prevail in the coolers of many meat retailers.

Six hams were sharp frozen in brine and stored at approximately —12°C.(10°F.) for four months. The hams were then thawed and two were taken for analysis to determine the effect of curing and frozen storage. The remaining four were smoked and then chilled for 24 hours. Two were taken for analysis and the other two were stored at 2 to 3°C.(35 to 38°F.) for 10 days before sampling.

Four hams were boned-out, given a light smoke, pressed into metal forms, and then cooked in vats along with other ready-to-serve hams. Two of the cooked hams were sampled after being chilled and the other two after 10 days' storage at 2 to 3°C. (35 to 38°F.).

The data collected in this experiment are presented (Tables 1 and 2).

Experiment 2: This experiment was undertaken to confirm and to extend Experiment 1 and was conducted similarly except that the whole hams rather than only center slices were used in this study. Three pairs of hams were studied, one pair being used to determine the losses in curing and smoking and the other two for determining the losses incurred in preparing ready-to-eat hams. The processing schedule for the smoked ham was the same as indicated above. The ready-to-eat hams were cured and smoked in a similar manner and then held at gradually increasing temperatures [up to 71°C.(160°F.)] in ovens with controlled humidity for at least 30 hours.

Experiment 3: To determine the loss of vitamins during the roasting of fresh and cured hams, four pairs of companion hams were used. Each of the left hams was skinned, boned, weighed, ground and mixed thoroughly, then frozen and stored at —29°C.(—20°F.) until the paired ham was ready for analysis. Two of the right hams were cooked as fresh hams in the Research Laboratory kitchen to an internal temperature of 85°C. (185°F.), then sampled for analysis. The other two were cured and smoked in the factory, cooked as smoked hams to an internal temperature of 74°C.(165°F.), and then sampled. These temperatures are the ones to which fresh and smoked, hams are customarily cooked (Committee on

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[1] Containing salt, sugar, nitrate, and nitrite in proportions conforming to U. S. Department of Agriculture, Bureau of Animal Industry regulations.

TABLE 1
Vitamin Content of Fresh and Processed Hams

Pair No.	Treatment	Weight		Protein		Thiamin		Riboflavin		Niacin	
		Fresh	Treated	Fresh	Treated	Fresh	Treated	Fresh	Treated	Fresh	Treated
	FIRST EXPERIMENT	kg.	kg.	pct.	pct.	µg./gm.	µg./gm.	µg./gm.	µg./gm.	µg./gm.	µg./gm.
1A	Cured	5.01	5.77	19.88	16.94	12.03	9.71	2.81	2.38	65.7	53.9
1B		5.94	6.65	19.00	16.56	10.15	8.88	2.13	1.84	37.5	31.5
3A	Cured, smoked, and chilled	6.82	7.22	19.69	18.38	10.04	8.13	2.18	1.81	50.8	44.0
3B		5.68	5.68	19.94	17.94	14.99	11.36	2.34	1.95	68.9	55.9
5A	Cured, smoked, and chilled; stored 10 days at 3°C.	6.31	6.48	21.00	20.06	13.74	11.25	2.22	1.93	52.1	46.5
5B		5.44	5.41	20.56	19.88	14.76	12.48	3.01	2.57	81.9	67.2
6A	Cured, smoked, and chilled; stored 30 days at 3°C.	7.11	7.13	19.25	18.94	11.15	9.48	2.57	2.36	49.1	42.0
6B		4.89	4.94	20.56	20.44	15.22	12.48	2.68	2.41	67.1	61.2
7A	Cured, frozen, stored 4 months at −12°C, thawed	6.28	7.34	19.56	16.88	9.74	7.96	2.52	2.11	62.7	46.8
7B		5.80	6.83	19.50	16.56	11.24	9.05	2.64	2.27	59.2	42.4
8A	Cured, frozen, stored 4 mo. at −12°C, thawed, smoked, and chilled	5.57	5.44	20.56	19.56	12.97	10.05	3.13	2.70	78.1	58.4
8B		6.76	6.71	20.13	19.25	16.72	12.20	2.77	2.57	85.4	52.7
9A	Cured, frozen, stored 4 mo. at −12°C, thawed, smoked, chilled, then stored 10 days at 3°C.	6.54	6.26	20.50	20.06	13.44	8.91	2.83	2.72	79.8	50.0
9B		5.29	5.01	19.63	19.81	11.34	8.47	3.17	2.68	70.0	55.4
2A	Cured, smoked, cooked as boiled ham, chilled	7.38	4.54[2]	20.06	21.81	14.75	11.33	2.80	2.91	55.8	44.1
2B		6.88	4.28[2]	19.88	22.19	9.32	7.15	2.82	2.60	75.8	56.1
4A	Cured, smoked, cooked as boiled ham, chilled, then stored 10 days at 3°C.	6.88	4.26[2]	19.63	21.56	12.62	9.32	2.47	2.37	71.9	54.5
4B		6.53	3.89[2]	19.38	21.23	13.96	10.42	2.36	2.18	54.9	41.0
	Curing pickle[1]	......	......	0	0.75	0	0.96	0	0.20	0	12.0
	SECOND EXPERIMENT										
10	Cured, smoked, and chilled like 3A	7.53	7.79	16.02[3]	16.82	7.68	6.82	1.98	2.13	46.6	43.6
11A	Cured, smoked, cooked as ready-to-eat ham	7.58	7.11	14.09[3]	15.64	10.78	7.85	1.84	2.24	40.1	36.5
11B		6.59	6.03	15.89[3]	19.57	10.03	6.60	2.05	2.53	41.5	41.6
	Curing pickle[1]	......	......	0	0.88	0	1.56	0	0.11	0	11.0

[1] Sample from the tierce in which the hams had been cured. [2] Bone removed. [3] In this experiment fresh hams were pumped with curing pickle before sampling, thus corresponding exactly to the hams going into cure.

Preparation Factors, 1942). It is to be noted that smoked hams require less cooking than the fresh products owing to previous heat treatments of the former.

Analytical Methods: In each case samples of the raw hams were frozen and stored at —29°C. until the corresponding processed sample was ready for assay. This eliminated variations in analytical conditions.

Samples were prepared for analysis as described by Rice, Beuk, Daly, and Robinson (1945). Thiamin analyses were made by the thiochrome procedure (Method 3) of Hennessy and Cerecedo (1939). Riboflavin and niacin were determined microbiologically upon enzyme-digested aliquots of the sample, Cheldelin, Eppright, Snell, and Guirard (1942). In addition, the riboflavin values in Experiment 2 were checked by fluorometric analyses, according to Peterson, Brady, and Shaw (1943). The cooking of meat frequently causes it to show a slight increase in riboflavin. Whether this indicates incomplete extraction from the raw samples or conversion of some precursor to riboflavin during cooking is unknown, although many investigators have observed and studied the phenomenon. Increases of this type have not been observed with other vitamins. Hence, small changes in riboflavin values are not as significant as equal changes of thiamin or niacin. The vitamin values shown (Table 1) are averages of duplicate assays differing by no more than 10 per cent.

The vitamin content per gram of protein has been used as the basis for calculating the retention of the vitamins in Experiment 2 (Table 2). This method of calculation eliminates any need for consideration of the changes in weight which the samples undergo since only negligible amounts of nitrogen are lost during processing. In Experiment 2 the method of "totals" was also used in calculating vitamin retention. In this type of calculation the vitamin content per gram of the control sample was determined and assumed to be the initial potency of the paired, processed ham. Multiplication of the potency by the initial weight of the experimental ham then gave the total initial vitamin content. After processing, the vitamin potency was again determined, multiplied by the processed weight, and the total final vitamin content was compared with the initial content. The method of totals was also used in Experiment 3.

<div style="text-align:center">DISCUSSION</div>

On the whole, there is an excellent retention of the vitamins during the curing of hams, there being only one to five per cent losses (Table 2). These losses are at least partially due to leaching of the vitamins into the curing pickle, as is indicated by the presence of small amounts of the vitamins in the pickle. Even when the hams are smoked. there are but slight losses, the greatest being 15 to 20 per cent of the thiamin. Storage of smoked cured hams up to 30 days at 3°C.(38°F.) had no detectable effect upon vitamin content.

The hams which had been cured, frozen, and stored at —12°C.(10°F.) for four months lost some niacin (15 to 20 per cent), but no thiamin or riboflavin. Upon subsequent smoking or storage after smoking, however, thiamin was not retained as well as in hams that had not been frozen.

Cooking of hams either ready-to-serve (smoke plus dry heat) or to a boiled stage (smoke plus water cook) naturally resulted in greater losses

TABLE 2

Retention of Vitamins During Processing and Cooking of Hams

Pair No.	Treatment	Thiamin µg. per gm. protein Fresh	Treated	Retained pct.	Riboflavin µg. per gm. protein Fresh	Treated	Retained pct.	Niacin µg. per gm. protein Fresh	Treated	Retained pct.
	FIRST EXPERIMENT									
1A	Cured	60.6	57.3	95	14.13	14.01	99	330	318	96
1B		53.5	53.6	98	11.19	11.11	99	197	190	96
3A	Cured, smoked, and chilled	51.0	44.2	87	11.08	9.86	89	258	239	93
3B		75.2	63.4	84	11.73	10.87	93	345	311	90
5A	Cured, smoked, and chilled; stored 10 days at 3°C.	65.4	56.2	86	10.57	9.59	91	248	232	93
5B		71.8	62.9	88	14.64	12.92	88	398	338	85
6A	Cured, smoked, and chilled; stored 30 days at 3°C.	58.0	50.2	86	13.36	12.47	93	255	222	87
6B		74.2	60.9	82	13.06	11.76	90	327	299	92
7A	Cured, frozen, stored 4 months at −12°C, thawed	49.8	47.2	95	12.89	12.50	97	344	277	81
7B		57.7	54.6	95	13.51	13.71	101	303	256	84
8A	Cured, frozen, stored 4 mo. at −12°C, thawed, smoked, and chilled	63.1	51.4	81	14.52	13.80	95	350	298	85
8B		83.0	63.4	77	13.78	13.31	97	424	274	65
9A	Cured, frozen, stored 4 mo. at −12°C, thawed, smoked, chilled, then stored 10 days at 3°C.	65.4	44.9	68	13.79	13.51	98	384	293	76
9B		57.8	42.8	74	16.12	13.51	84	356	279	78
2A	Cured, smoked, cooked as boiled ham, chilled	73.6	52.0	71	13.90	13.31	90	278	202	73
2B		46.8	32.2	69	14.80	11.86	83	386	253	66
4A	Cured, smoked, cooked as boiled ham, chilled, then stored 10 days at 3°C.	64.4	43.3	67	12.47	11.00	88	366	253	69
4B		72.0	48.2	68	12.16	10.24	84	283	193	68

SECOND EXPERIMENT

Pair No.	Treatment	Total thiamin content Fresh mg.	Treated mg.	Retained pct.	Total riboflavin content Fresh mg.	Treated mg.	Retained pct.	Total niacin content Fresh mg.	Treated mg.	Retained pct.
10	Cured, smoked, and chilled like 3A	47.8	40.5	85	12.35	12.68	103	291	259	89
11A	Cured, smoked, cooked as ready-to-eat ham	76.6	50.2	65	13.06	14.31	110	285	233	82
11B	Cured, smoked, cooked as ready-to-eat ham	63.3	33.7	53	12.90	12.92	100	261	212	81

Pair No.	Treatment	Total thiamin content Fresh mg.	Treated mg.	Retained pct.	Total riboflavin content Fresh mg.	Treated mg.	Retained pct.	Total niacin content Fresh mg.	Treated mg.	Retained pct.
10	Cured, smoked, and chilled	53.13	43.57	82	13.69	13.60	99	322.2	278.1	86
11A	Cured, smoked, cooked as ready-to-eat ham	69.04	46.10	67	11.79	13.17	112	256.4	214.2	84
11B		57.37	30.48	53	11.69	11.71	100	236.7	192.0	81

of vitamins than the curing and smoking operations. The greater losses of riboflavin and niacin in the boiled hams (Samples 2 and 4) may well be accounted for by leaching of these vitamins into the water in which the hams were cooked, even though most of the meat was covered with tight-fitting forms which tended to minimize contact of the meat with water.

The retentions listed (Table 2) are based on micrograms of vitamins per gram of protein and thus may be one to two per cent high, since this fraction of the nitrogen of the hams was found in the curing pickle. In Experiment 2 the values based on micrograms of vitamin per gram of protein agree closely with those calculated from the total vitamin contents of the raw and processed hams. The two sets of values for the three samples, on nitrogen and totals basis, are, respectively: for thiamin 85 and 82, 65 and 67, 53 and 53; for riboflavin, 103 and 99, 110 and 112, 100 and 100; and for niacin, 89 and 86, 82 and 84, 81 and 81.

As pointed out by Schweigert, McIntire, and Elvehjem (1944) retentions calculated from the total vitamin content of a raw sample and that of a carefully paired sample after processing do not include errors owing to losses of nitrogen during processing. However, unless the samples are exactly paired with respect to both weight and composition (protein, fat, moisture, vitamins), errors of equal or greater magnitude may be introduced. The method of calculation must, therefore, be carefully considered in all work involving paired samples.

Averages of the retentions for the cured hams (including the stored samples since there was no detectable loss during the storage period) are in the same range as those reported on a nitrogen basis by Schweigert et al. (1944), their values being 85, 104, and 106 per cent for thiamin, riboflavin, and niacin, respectively, as compared with averages of 85, 93, and 90 per cent (Table 2).

In so far as conservation of nutrients is concerned it would seem that the curing and smoking of hams might entail unnecessary losses of nutrients. Actually, however, the effect of these two operations is such that smoked cured hams require a less severe home-cooking schedule, thereby compensating for losses of vitamins occurring during commercial processing. This was demonstrated in Experiment 3, in which the vitamin content of cooked raw hams was compared with that of cooked smoked hams, each being cooked according to the schedules recommended for the two types of meat. The retentions of thiamin and niacin in the cooked smoked hams were greater than those of cooked fresh hams, but the riboflavin retentions were higher for the latter type. In view of the uncertainties in riboflavin analyses of cooked meats, it is doubtful if the differences found for that vitamin are significant. Likewise, the slightly larger thiamin retention in the cooked smoked hams might be due to experimental variation.

SUMMARY

Several experiments involving a total of 50 hams have been conducted to determine the effects of curing, smoking, cooking, and storage upon the thiamin, riboflavin, and niacin contents of hams.

TABLE 3

Retention of Vitamins During Cooking of Fresh and Smoked Cured Hams

Ham No.	Treatment	Weight		Thiamin					Riboflavin					Niacin				
		Fresh	Cooked	Fresh	Cooked	Total Fresh	Total Cooked	Retained	Fresh	Cooked	Total Fresh	Total Cooked	Retained	Fresh	Cooked	Total Fresh	Total Cooked	Retained
		gm.	gm.	μg./gm.	μg./gm.	mg.	mg.	pct.	μg./gm.	μg./gm.	mg	mg.	pct.	μg./gm.	μg./gm.	mg.	mg.	pct.
12 R&L	Cooked as fresh ham[1]	5350	3250	9.82	6.98	52.5	22.35	42	2.72	3.89	14.54	12.63	87	27.3	24.7	145.8	80.2	55
12 Br.	Broth from 12R	......	412	......	11.15	......	4.60	51[2]	......	4.81	......	1.97	100[2]	......	61.9	......	24.2	72[2]
13 R&L	Cooked as fresh ham[2]	5515	3450	9.89	5.45	54.5	18.8	35	2.86	4.02	15.76	13.80	88	38.1	35.1	209.5	121.5	58
13 Br.	Broth from 13R	......	625	......	8.04	......	5.0	44[2]	......	4.08	......	2.56	104[2]	......	75.3	......	47.0	80[2]
14 R&L	Cured and smoked, then cooked[3]	6095	4045	6.12	3.93	37.3	15.9	43	2.64	3.08	16.09	12.18	76	33.6	32.6	181.0	124.0	69
14 Br.	Broth from 14R	......	368	......	1.94	......	0.7	45[2]	......	4.78	......	1.76	87[2]	......	80.6	......	29.6	85[2]
15 R&L	Cured and smoked, then cooked[3]	5680	4002	10.60	7.55	60.3	30.2	50	3.07	3.27	17.43	13.10	75	42.0	44.0	238.8	166.9	70
15 Br.	Broth from 15R	......	496	......	10.71	......	5.3	58[2]	......	3.79	......	1.88	86[2]	......	71.5	......	36.21	85[2]

Averages:

	Thiamin	Riboflavin	Niacin
Cooked as fresh ham, meat only	39	87	56
Cooked as fresh ham, meat plus broth	47	102	76
Cooked as cured and smoked ham, meat only	46	75	69
Cooked as cured and smoked ham, meat plus broth	51	86	85

[1] To internal temperature of 185°F. [2] Includes vitamin content of ham plus broth. [3] To internal temperature of 165°F.

Losses during sweet-pickle curing are negligible. During curing and smoking only eight to 15 per cent of the vitamins are lost, but production of either ready-to-eat or boiled hams involves greater losses—up to 30 to 40 per cent of the thiamin and 20 to 30 per cent of the niacin. Freezing and freezer storage do not greatly reduce vitamin content but do predispose to greater losses during subsequent smoking operations.

The curing and smoking of hams condition the meat so that less severe cooking conditions are needed than for fresh hams, thereby reducing losses during home cooking. Consequently a cooked, cured, and smoked ham retains as much of its initial vitamin content as a cooked fresh ham.

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A RAPID METHOD FOR ESTIMATION OF WHEY PROTEINS AS AN INDICATION OF BAKING QUALITY OF NONFAT DRY-MILK SOLIDS [1]

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The use of nonfat dry-milk solids in the bread-baking industry has become very important during the past 15 years. The importance of this product to the baking industry has been largely brought about by improvements in the manufacturing process of milk solids. It was found necessary to preheat the separated milk to control water absorption of the dough and to produce maximum loaf volume. There are no published routine methods for estimating baking quality of dry milk except those involving experimental baking or other somewhat laborious dough-testing methods. The object of this investigation was to find a rapid method for determining the baking quality of nonfat dry-milk solids.

The turbidity method described in this paper is based on the longer procedure of Harland and Ashworth (1945) involving the use of sodium chloride and hydrochloric acid for the quantitative estimation of the whey proteins of separated milk. Hawk and Bergeim (1937) described a method for the quantitative determination of albumin in urine based on the turbidity of the albumin following treatment with sulfosalicylic acid. Zeleny (1941) developed a simple method for the turbidimetric estimation of gluten in wheat flour.

DEVELOPMENT OF METHOD

Procedure: One gram of nonfat dry-milk solids, four grams of sodium chloride,[3] and 10 ml. of water are shaken vigorously for approximately 40 seconds in a stoppered test tube. The tube is immersed in a water bath maintained at approximately 40°C.(104°F.) for a period of 20 to 30 minutes. Without further agitation, except that necessary to permit pouring, the precipitated casein is filtered off on a nine-cm. S. and S. No. 604 paper (Whatman paper Nos. 1 and 2 are also satisfactory.) A one-ml. aliquot of the clear filtrate is diluted in an Evelyn tube with nine ml. of saturated sodium chloride solution. Duplicate dilutions may be made. The sample is placed in the Évelyn colorimeter fitted with a 420-millimicron filter and the instrument adjusted to read 100 per cent transmittance. After removing the tube, the center reading is recorded. Two drops of 10 per cent hydrochloric acid are added to the tube, and after

mixing three or four times during a period of 10 minutes, the tube is returned to the colorimeter (which has been set at the recorded center reading) and the per cent transmittance of the turbid solution is determined. The milligrams of whey protein per milliliter of the filtrate may be obtained from a calibration curve or table. This value multiplied by the factor 12.0 gives the whey protein N per gram of nonfat dry-milk solids. The factor 12.0 is the result of determining the volume of filtrate equivalent to the milk solids both volumetrically and gravimetrically. When 10 ml. of fresh separated milk are used in place of the milk solids and water, a factor of 1.11 is used to convert to mg. N per ml. of milk.

TABLE 1

Preparation of Standard Wheys From Separated Milk

Heat treatment of milk	Non-casein N	Non-protein N	Whey protein N
	mg./ml.	*mg./ml.*	*mg./ml.*
None	1.015	.271	.744
Held at 75°C.(167°F.) for 20 min	0.550	.274	.276
Held at 95°C.(203°F.) for 5 min	0.351	.276	.076

Calibration Curve: Fresh separated milk was used for the standardization of this method. The casein-free filtrates were prepared and N determinations (Table 1) were made according to the method of Harland and Ashworth (1945). The filtrate was diluted 1-10 with saturated sodium chloride solution and suitable aliquots were transferred to the Evelyn tubes for making up to the 10-ml. volume for development of turbidity. In order to investigate the possibility of the more heat-stable fractions of the whey proteins having a different turbidity per unit of N, fresh separated milk was heat-treated at 75°C.(167°F.) for 20 minutes and a second sample at 95°C.(203°F.) for five minutes. Suitable dilutions were made and the turbidities produced by the whey from heated milk were found to fall on the same standardization curve (Fig. 1) as produced by the unheated milk. The 420-millimicron filter was selected for this determination since it gave a lower per cent transmittance for the turbid solution than other available filters having wave lengths up to 660 millimicrons.

Development and Stability of Turbidity: A large sample of the sodium chloride-milk filtrate was prepared for this study. It was soon discovered that frequent shaking of the mixture following the addition of the hydrochloric acid gave much better results than agitation only at the beginning and the end of the time allowed for precipitation of the whey proteins. Not more than 10 minutes were required for the development of maximum turbidity in each of four samples of nonfat dry-milk solids having variable whey protein content. The turbidity was found to remain constant for at least 40 minutes following agitation.

Effect of Casein Precipitation Procedure: The effect of time and temperature of sodium chloride precipitation of casein from the milk on the turbidity produced by the whey proteins is shown (Table 2). Since 20 minutes of standing was found to be about the minimum for rapid filtration, that time was used except in the one case when the mixture was

allowed to stand for 16 hours at room temperature. A temperature of 40°C. was chosen as the best of the series since at this temperature the non-casein filtrate was clear, the filtration was rather rapid, and the change of the amount of whey protein with temperature was small, between 35 and 45°C.(95 and 113°F.).
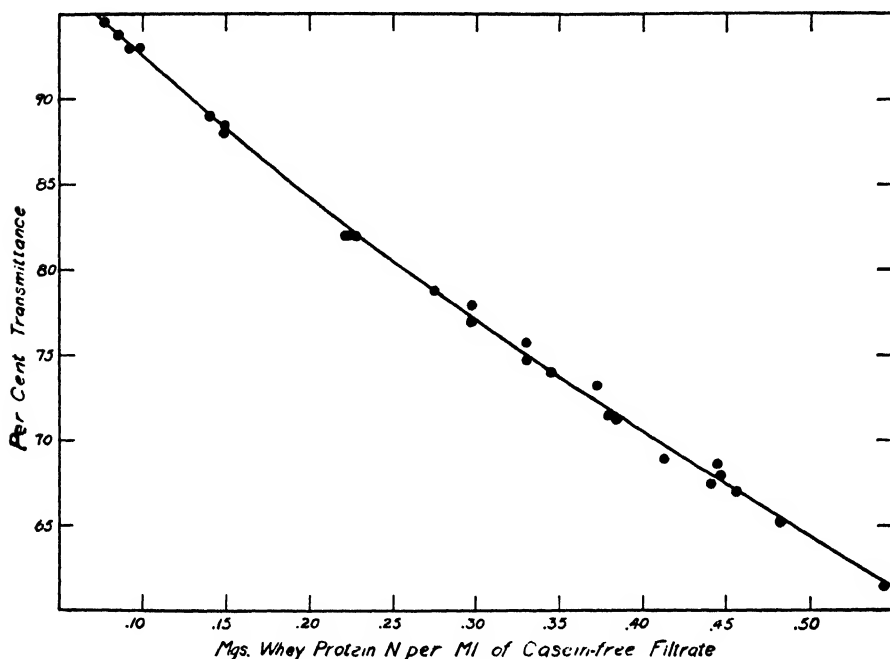

Fig. 1. Standard curve.

Reproducibility: The reproducibility of the method was found to be satisfactory; for example, a series of six replicate determinations made together showed a maximum deviation from the mean of about three per cent. The variation was no greater for a series of five aliquots analyzed on different days.

Effect of Time and Temperature of Precipitation of Casein on Turbidity of Precipitated Whey Proteins

Temperature	Time	Whey protein N per gram of milk solids
°C.	min.	mg.
21	16(hr.)	3.95
21	20	4.47
35	20	3.77
40	20	3.75
45	20	3.69
50	20	3.52
55	20	3.48
60	20	3.37

APPLICATION OF METHOD

The whey protein N was determined for 18 good and 18 poor baking samples; the latter included 17 samples of nonfat dry-milk solids and one sample of fresh skim milk (Table 3). The good baking samples are arranged in order of their decreasing whey protein content and the poor baking samples in order of their increasing whey protein content.

TABLE 3

Relation of Whey Protein Content to Baking Quality of Nonfat Dry-Milk Solids

Good baking quality		Poor baking quality	
Sample No.	Whey protein N per gram	Sample No.	Whey protein N per gram
	mg.		*mg.*
122	2.12	105	0.99
8	2.07	119	1.13
110	1.89	116	1.24
121	1.40	104	1.86
111	1.27	24	1.86
3	1.24	101	1.97
10	1.14	30	2.05
23	1.12	115	2.33
12	1.07	108	2.38
17	1.07	114	3.10
126	0.99	117	3.35
21	0.99	112	3.63
28	0.88	106	3.68
5	0.86	113	3.68
129	0.83	135	4.40
132	0.80	136	6.43
2	0.80	134	6.59
103	0.67	Fresh skim	7.65

DISCUSSION

The turbidimetric method described in this paper for the estimation of whey proteins in fresh separated milk as well as nonfat dry-milk solids is reasonably accurate, rapid, and requires very little equipment in addition to a colorimeter. The results obtained by this method were not compared with other generally accepted methods for whey proteins since it is based on a new procedure described by Harland and Ashworth (1945). The latter paper compares the use of sodium chloride and hydrochloric acid with a generally accepted method for the quantitative estimation of whey proteins.

The baking quality of the milk samples was determined by commercial laboratories although in most cases their results were confirmed by baking tests in our own laboratory. The chief criteria of quality are water absorption of the dough and loaf volume. The usual level of nonfat milk solids for testing is six to eight per cent of the weight of the flour. By assuming all samples of nonfat dry-milk solids having 1.41 or more milligrams of whey protein N per gram to be of poor baking quality, the method would have been a reliable means of predicting the baking quality of 29 out of

a total of 35 samples (Table 3). There would necessarily be borderline cases in any such a test as there is no means of making a sharp differentiation between good and poor baking, nonfat, dry-milk solids. The results show that all samples having over 2.2 mg. of whey protein per gram are of poor baking quality. Several replicate bakes were made on Samples 105, 119, 122, and 110. Although the absorption was good on Samples 105 and 119, the dough was somewhat sticky from the mixer and the loaf volumes were below normal. The absorption was slightly below normal for Samples 122 and 110 but otherwise the baking quality was satisfactory.

The data (Table 3) show that Samples 134 and 136 have had very little heat treatment as the whey protein N for these samples is 84 to 85 per cent of that present in the equivalent amount of solids of fresh skim milk.

SUMMARY

A turbidimetric method for whey proteins in milk is described. The method involves the sodium chloride precipitation of the casein and acidification of the diluted filtrate to produce the turbidity. The latter is measured by determining the per cent transmittance of the turbid suspension of whey proteins.

Since the method is relatively simple, accurate, and rapid, it is adapted to routine determinations of whey proteins in milk. By selecting an arbitrary value for whey proteins as a reference point, 29 out of a total of 35 samples of good and poor baking, nonfat, dry-milk solids could be properly classified.

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STABILITY OF ASCORBIC ACID IN VARIOUS CARRIERS

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On freely selected diets containing an ample supply of fresh fruits and vegetables, the attainment of the daily ascorbic acid requirement is a relatively simple matter. Should the economic status of an individual limit the purchase of fresh fruits and vegetables, the diet would probably still furnish adequate ascorbic acid in the form of potatoes and cabbage, "the poor man's food." When one turns to military rations, however, the problem of obtaining even small quantities of ascorbic acid is not as easily solved as that of the poor man. The military characteristics of operational rations, such as the C, K, and 10-in-1, permit the selection of only a relatively narrow range of food products. Most of these are deficient in ascorbic acid, and the remainder tend to undergo severe losses on long storage in unfavorable climates such as may be expected in global warfare. Considerable effort, therefore, has been devoted to a search for a variety of stable carriers, both natural and synthetic, to supply the daily intake of ascorbic acid as well as to meet other military requirements.

In the early work, fortification experiments were limited to those components which appeared to be natural carriers of ascorbic acid, such as fruit-juice beverages and fruit-flavored hard candy. Since very few such stable carriers could be found, it was necessary to provide a large proportion of the daily ascorbic acid requirement in each source. As more information on the likes and dislikes of Army personnel became available, fortification work spread to unnatural carriers (i.e., cocoa and coffee beverage, etc.) with the result that a greater number of stable carriers became available. It was thus possible to decrease the amount of ascorbic acid added to each carrier. The intention was to employ three or four ration items to furnish the daily ascorbic acid requirement, thus eliminating the possibility that the soldier would get no ascorbic acid owing to the dislike of a single fortified item.

EXPERIMENTAL PROCEDURE

The method used in setting up the stability tests was, in general, similar for all commodities studied.[2] Vitamin fortification was added at an appropriate stage of processing to samples prepared in accordance with the commercial practices which were ordinarily employed in the manufacture of such items. When the fortified product was received, a large number of samples were assayed to determine the distribution of ascorbic

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[1] Formerly Quartermaster Corps Subsistence Research and Development Laboratory.
[2] This plan as outlined was followed as closely as possible for every item studied. During the early history of the laboratory, a few exceptions occurred owing to the non-availability of sufficient samples and lack of incubator space.

TABLE 1

Retention of Ascorbic Acid in Carriers Studied

Description of carrier	Number of exp. included	Fortification	Retention						Moisture content
			3 months		6 months		12 months		
			R.T.[1]	100°F.	R.T.	100°F.	R.T.	100°F.	
			pct.	pct.	pct.	pct.	pct.	pct.	pct.
Beverages and beverage bases									
1. Fruit beverages									
a. Synthetic sources									
Lemon powder synthetic	4	60 mg. ascorbic acid/7 gm.	90-100	90-100	90-100	90-100	90-100	90-100	1.5
Orange powder synthetic	1	60 mg. ascorbic acid/7 gm.							
Grape powder synthetic	1	50 mg. ascorbic acid/7 gm.							
b. Natural sources									
Deh. orange-juice powder, Type 1	3	None	100	60	100	34	......[2]	......	1-2
Deh. orange-juice powder, Type 2	1	None				60		20	1-2
Canned orange juice	2	None	90	80	95	60			86
c. Combination of natural and synthetic sources									
Liquid orange beverage base	2	80 mg./8 oz. serving	66	39	40	14			80
Apple juice	1	35 mg./100 c.c.	95	75	95	50			88
2. Other beverages									
Cocoa beverage powder, bulk	4	30 mg. ord. as. acid/1½ oz. bulk	85-100	85-100	85-100	85-100			4.0
		30 mg. stab. as. acid	85-100	85-100	85-100	85-100			
Cocoa beverage powder, disc	4	30 mg. ord. as. acid/disc.	90-100	85	85-100	60-70			
		30 mg. stab. as. acid/disc.	90-100	85	85-100	65-75			
	1	25 mg. stab. as. acid/5 gm.	85	85	85	85			2.0
Soluble coffee product	1	30 mg. ord. as. acid/5 gm.	90	90	90	90			
Candy and confections									
1. Hard candy									
a. With citric acid									
Packages of 10	5	25 mg./package	90-100	90-100	90-100	90-100	......	85-100	1
Ind. wrapped pieces	10	1.5 -20 mg./piece	......	90-100	......	80-100	......	80-100	1
Unwrapped pieces	1	15 mg./piece	98	98	90	90	90	90	
b. Without citric acid									
Butterscotch	2	10-20 mg./piece	90-100	90-100	85-100	85-100		90-100	1
Pressed candy	5	10-20 mg./piece	85-100	85-100	85-100	85-100			1
2. Soft candy									
Lozenges	1	10 mg./piece	......	35	......	30			4
Mints	1	20 mg./piece	85	73	85	54			6

Product	No.	Specification							
K ration caramels	1	10 mg./piece	......	41	80	30	50	30	8
Chocolate	3	40-80 mg./1-oz. bar	56	40	30	30	45	......	2.5
Starch-jelly confection	1	20-80 mg. ord. as. acid/2-oz. bar	60	30	......	......	......	......	17-22
	1	40 mg. stab. as. acid/2-oz. bar	90	75	90	60	......	......	17-22
Jelly beans, pan coated	1	45 mg./0.6 oz.	......	95	......	80	......	52	17
3. Sugar, granulated									
Discs	2	30 mg./1.6-oz. disc	85-100	85-100	75-85	75-85	......	......	1
Bulk	2	30 mg./1.6 oz.	95-100	95-100	95-100	95-100	......	......	1
Gum									
Stick	4	10 mg./stick	......	33	......	10	......	......	......
Candy-coated									
As. acid in center	3	25 mg./piece	......	56	......	40	......	32	3
As. acid in center	1	9 mg./comb. of sod. ascorbate plus l-ascorbic acid	60	90	......	50	......	......	3
As. acid in coating	1	Appr. 40 mg./6 pieces	75	65	70	45	......	......	3
Jams and jellies									
Orange jelly, 4¼-oz. can	1	2 mg./gm. jelly	......	40	......	......	......	......	......
Grape jelly, 1½-oz. can	1	30 mg./oz.	60	34	50	20	......	......	30
Assorted jams, 1½-oz. can	11	30 mg./oz.	......	35	......	......	......	......	30
Jams, 11-oz. can, assorted	10	70 mg./100 gm.	......	50	......	20	......	......	......
Assorted jams in aluminum tubes, 1½-oz.	9	30 mg./oz.	......	......	......	......	......	......	......
Jams in guava	10	10% of weight of fruit	......	0-25	......	20	......	......	30
Jams without guava	10	30 mg./oz.	......	47	......	17	......	......	30
Cereal and baked products									
Bulk premixed cereal	8	30-60 mg./2 oz. cereal	90	89	95	81	80	78	3-5
Disc premixed cereal	4	30-75 mg./2-oz. disc	74	69	78	61	80	50	3-5
Fruit cake	3	60 mg./100 gm.	90[3]	......	......	......	......	......	
Cereal fruit bar	2	75 mg./4-oz. bar	......	36	......	20	100	......	
Sandwich cookies, Type 1	1	30 mg./cookie	100	100	100	......	......	90	
Sandwich cookies, Type 2	2	30-50 mg./cookie	80	55	......	96	......	......	
Miscellaneous products									
Fruit bar	2	45 mg./2-oz. bar; guava to furnish 45 mg. as. acid	23	10	......	10	......	......	18-20
Gelatin	3	4 gm./100 gm.	65	62	65	62	......	......	......
Cheese spread with ham and relish	2	50 mg./3⅞-oz. can	88	55	78	18	......	......	......

[1] R. T. = room temperature. [2] Indicates that these experiments have been completed and no further data will be forthcoming. Blank spaces indicate that these experiments are in progress and further data will be forthcoming. [3] Ninety percent lost on baking.

acid from unit to unit and also to establish a representative initial value. The fortified samples and similar unfortified samples were then packaged in a manner identical to or closely resembling that in which they appeared in the rations along with the other ration items. The packaged samples in ration containers were then divided to be stored at room temperature and 37.8°C.(100°F.), humidity not controlled. In some instances other storage temperatures, such as —19.4, 1.7, 43.3, 48.9, and 55° C.(—3, 35, 110, 120, and 131°F.), were used in addition to the room temperature and 100°F. storage temperature. At frequent time intervals, ranging from fortnightly to monthly and bimonthly, samples were removed from each storage temperature, assayed for ascorbic acid and examined for changes in palatability and moisture content. The number of samples removed from each storage temperature at each time interval was determined by the extent of the range of the initial ascorbic acid content in a particular product and usually was from one to five. The procedure for determining reduced ascorbic acid was essentially the photometric dye-reduction method as modified by Loeffler and Ponting (1942). Assays for dehydro and total ascorbic acid content of many items were made frequently but not as regularly as the assays for reduced ascorbic acid. Both the phenylhydrazine, Roe and Oesterling (1944), and H$_2$S, Bessey (1938), methods were used for the dehydro and total ascorbic acid.

The accumulated periodic results were then used to ascertain the percentage retention of ascorbic acid at the different storage temperatures. These calculations were based upon the amount of ascorbic acid which was found in the product in the initial analyses. The difference between the initial value and the actual amount put into the product represented the processing loss. In dry products there were no demonstrable processing losses. For other carriers the processing losses could not always be calculated because the actual amount put in by the producer was not known. Oftentimes too, when few samples were available, it was difficult to decide whether a discrepancy between the initial assay and theoretical value constituted a processing loss or was due to poor distribution. It is for this reason that processing losses are not discussed in this paper. If a carrier retained from 75 to 80 per cent of the added ascorbic acid after six months' storage at 100°F., it was considered a stable carrier. This criterion was chosen to represent actual field storage times and temperatures, particularly in the Pacific Ocean areas. Although actual field storage temperatures are somewhat lower in almost all cases, the storage times are normally longer, and the test conditions accordingly are a somewhat accelerated representation. Since many foods undergo deteriorative changes above 100°F. which do not take place at an appreciable rate at lower temperatures, it was felt that a greater degree of acceleration in the test was not warranted.

RESULTS AND DISCUSSION

The types of products investigated were beverages and beverage bases; candy and confections, including gum; jams and jellies; cereals and baked products; gelatin; and meat and dairy products. Under each group of materials named above, many variations and modifications were tried (as can be seen in Table 1). A summary is given (Table 1) of the average

percentage retention of ascorbic acid in the various carriers after storage at room temperature and 100°F. for three, six, and 12 months, respectively. The 12-months' retention was not determined in all products. Information on moisture content and fortification are also shown (Table 1); the comparative stability of the various carriers is illustrated (Figs. 1, 2, 3, and 4). These plots are shown on semi-logarithmic co-ordinates, which will give linear relationships for first-order reactions. The slope of the line of best fit is a convenient expression of the reaction rate.

<center>BEVERAGES AND BEVERAGE BASES</center>

The carriers studied under this group were synthetic fruit-juice powders, natural dehydrated orange juice, canned orange and apple juice, and a liquid orange-juice beverage base which was a combination of a natural source and added synthetic ingredients. While the natural orange-juice powder and canned orange juice were not fortified, the synthetic citrus powders were fortified with approximately 60 mg. of ascorbic acid per seven-gram package and the grape powder with approximately 50 mg. per seven-gram package. The apple juice was fortified at the rate of 35 mg. per 100 c.c. of juice and the liquid beverage base had enough ascorbic acid added to furnish 80 mg. of ascorbic acid in an eight-ounce serving of the finished drink. A comparison of the stability of ascorbic acid in these carriers (Fig. 1) indicates that while the ascorbic acid in all synthetic powders had excellent stability, that in the natural sources and liquid beverage base was not as good. The natural dehydrated sources and canned
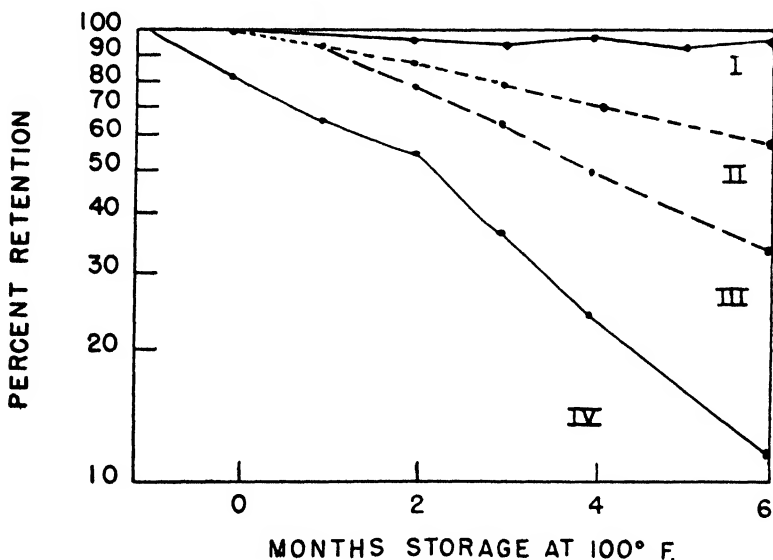
FIG. 1. Per cent retention of ascorbic acid in beverages and beverage bases on storage.

    I. Synthetic sources—fruit, coffee, and cocoa powder.
   II. Canned orange juice and some orange powders, II.
  III. Other orange powders, I.
  IV. Liquid beverage base.

*juices in turn showed better stability than the added ascorbic acid in the
liquid orange-beverage base.* The stability of the ascorbic acid in the
natural dehydrated sources also varied greatly, depending upon the type
of processing used in the preparation. The discrepancies between the
stability of ascorbic acid in the synthetic powders and natural dehydrated
sources are difficult to explain on the basis of known factors affecting the
stability of ascorbic acid such as pH, oxygen, and moisture content. Both
types of products were acid and of the same moisture content. The only
apparent differences between the natural dehydrated sources and the syn-
thetic powders were the added dextrose and citric acid present in the
latter, and the fact that the natural juice component of the synthetic
powder was mixed with high-dextrin corn syrup before drying. These
two substances may be concerned with stabilizing the ascorbic acid.

The palatability of these items seemed to be related to the vitamin
stability. The liquid beverage base developed a terpene odor and flavor,
darkened, and made a very poor drink. The natural products tested also
developed undesirable bitterness. The synthetic powders were quite pala-
table if served cold, and even at the end of one year's storage, although
there was slight weakening in the characteristic flavor, the beverages
made from them were satisfactory. Investigations were also made on the
stability of ascorbic acid added to other ration beverages, such as soluble
coffee product and cocoa beverage powder. Retentions of 80 to 90 per cent
were obtained after six months' storage at 100°F. The bulk form (that
packed in 1½-ounce bags) of cocoa beverage powder retained approxi-
mately 20 per cent more of the added ascorbic acid than did the cocoa
powder compressed as a two-ounce disc and wrapped in cellophane at
the end of this time. The preparation of beverage powders, as either the
hot or cold beverage, did not result in appreciable ascorbic acid losses.

CANDY AND CONFECTIONS

This group included several flavors of hard candy with citric acid and
one flavor (butterscotch) without citric acid; hard- and soft-pressed sugar
lozenges; and the softer confections, such as chocolate, caramel, and
starch-jelly candy. In general, the stability of added ascorbic acid in
these products was related to the type of structure into which the ascorbic
acid was incorporated as well as the acidity and moisture content of the
carrier. Those carriers which presented a hard impenetrable surface both
to oxygen and moisture because of their closely knit structures suffered
the least losses in ascorbic acid. The degree to which the external and
internal surfaces of other carriers afforded protection against moisture
and oxygen was a determining factor in the stability of the added ascorbic
acid. The moisture content was important not only because of its adverse
effect on the stability of ascorbic acid but also because of its influence on
the structure of the carrier. The beneficial effect of a more acid carrier,
on the other hand, was not its relation to the structure, but its ability
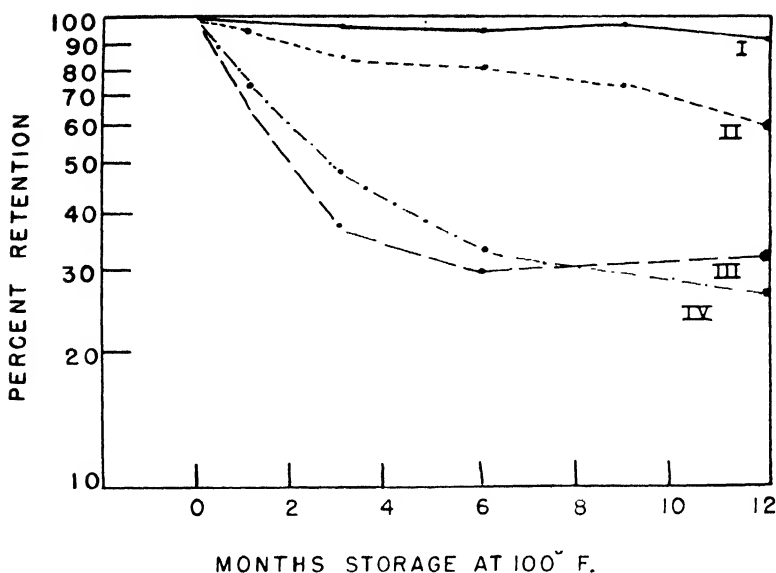to retard destructive changes of ascorbic acid in the presence of oxygen
and moisture.

MONTHS STORAGE AT 100° F.

Fɪɢ. 2. Per cent retention of ascorbic acid in candy and confections on storage.
    I. Hard candy containing citric acid.
    II. Other hard candy.
    III. Soft candy, chocolate, starch jelly confection, lozenges, etc.
    IV. Gum.

In line with the above statements, hard candy having a solid crystal structure retained 90 to 100 per cent of the added ascorbic acid for at least a year. Ascorbic acid in hard-pressed mints and candy wafers was more stable than in soft mint lozenges, as shown by their respective retentions of 85 and 40 per cent at the end of six months' storage at 100°F. Lemon-flavored pressed mints were better carriers than peppermint-flavored ones, the only differences, other than flavoring oils, between the two flavors being the acidity. Other soft candy proved to be as poor a medium for ascorbic acid as soft-pressed mints, as would be expected from the large amount of internal and external surface exposed to moisture and oxygen. Jelly beans, which were made of the same base as the starch-jelly confection, had much better stability (95 per cent retention, three months' storage at 100°F.) than the starch-jelly confection (30 per cent retention, three months' storage at 100°F.) because of the protection afforded the ascorbic acid by the thin, hard, exterior coating. Ascorbic acid added to granulated sugar was retained somewhat better in the bulk form than in the disc form, but both types of sugar were good carriers as judged by the criterion of 75 to 80 per cent retention following six months' storage at 100°F.

The palatability and acceptability of these candies as a whole was good. Although graining occurred in some of the hard candies and bloom developed on the surface of the chocolate bars at 100°F. storage temperature, these products remained satisfactory. The two-ounce starch-jelly confection bar hardened somewhat but also had good acceptance. The jelly beans retained their individual shape and showed no deterioration. In all candy containing citrus oils, a slight terpene flavor was evident after storage, but this did not detract seriously from the desirability of these products.

Gum: Several varieties of stick and candy-coated gum were tested in this category. Stick gum was one of the poorest carriers yet tested, regardless of its flavor. Candy-coated gum, with the ascorbic acid incorporated into the gum center at 25 mg. per tablet, showed better retention of ascorbic acid than the stick form; and the thicker the coating the better the retention, provided the coating did not crack. The incorporation of the ascorbic acid into the candy coating itself did not improve the stability of the ascorbic acid as evidenced by the poor retention after three months' storage at 100°F. (Table 1). The results obtained thus far on gum seem to point to the abandonment of this product as a carrier for ascorbic acid, not only from the standpoint of poor stability but also because of the poor quality of gum which results from the added acid.

JAMS AND JELLIES

Extensive studies on jams and jellies include data on approximately 30 varieties. Various levels of fortifications were studied along with the substitution of guava proportions for 10 per cent of the weight of the fruit for the express purpose of determining the effect of guava on stability of the added ascorbic acid. Although some varieties showed better retention of ascorbic acid at the various time intervals than others, no single jam retained sufficient ascorbic acid to be labeled a stable carrier.
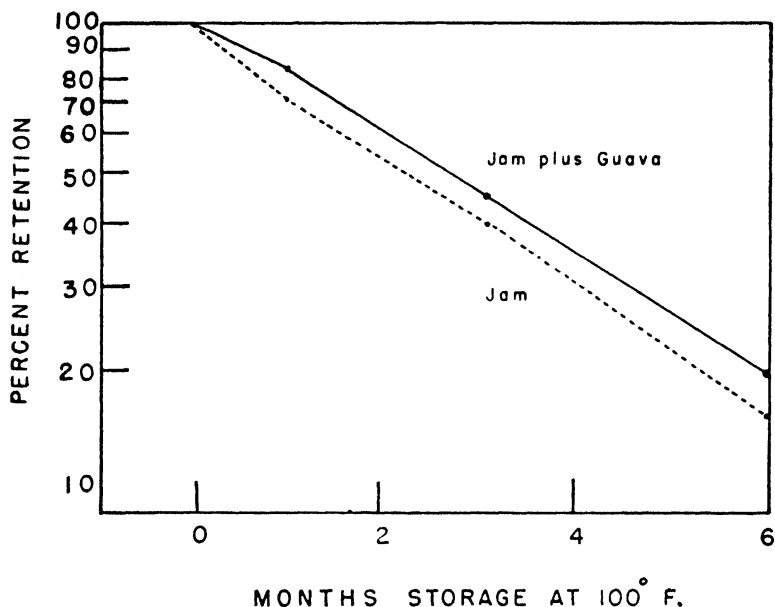

Fig. 3. Per cent retention of ascorbic acid in jams on storage.

At the end of three months' storage at 100°F., from 50 to 65 per cent of the added ascorbic acid activity had disappeared and only about 20 per cent remained at the end of six months. The addition of guava in the form of guava concentrate or guava purée did not improve the retention of ascorbic acid outside the range of experimental error permitted for the assay methods (Table 2).

TABLE 2

*Effect of Guava Addition on Retention of
Ascorbic Acid in Jams*

Storage time	Retention	
	With guava	Without guava
	pct.	pct.
1 mo., 100°F..	81	71
3 mo., 100°F..	47	40
6 mo.. 100°F..	20	17

Despite the fact that jams and jellies theoretically might appear to be excellent carriers of ascorbic acid because of their low pH and protection from oxygen, the destruction of added ascorbic acid is so great as to make jams a most undesirable carrier. All the jams tested had good acceptability even though there was a slight darkening in the color and a diminution of the characteristic flavor after three to six months' storage at 100°F. In the jams containing guava, the flavor of the guava was perceptible and the possibility exists that these jams would thus become undesirable to some tastes.

CEREAL AND BAKED PRODUCTS

The cereals examined comprised all the premixed[3] bulk cereal and some compressed cereal discs appearing in Army rations. The levels of fortification ranged from 30 to 75 mg. ascorbic acid per two ounces of cereal and included not only ascorbic acid of varying particle size but also ascorbic acid in the form of ground-up, fortified hard candy, fortified gelatin, and two commercial forms of stabilized ascorbic acid. In some of the studies on cereals it was difficult to interpret the data because of poor distribution of ascorbic acid. This would be expected in cereal because of the large granule size.

Despite the limitations mentioned the data obtained on these cereals (Table 3) indicate that:

a. Premixed bulk cereal is a more stable carrier than the disc cereal.

b. Premixed bulk cereal of Manufacturer C was not as stable as the other bulk cereals.

c. The stability of ascorbic acid in premixed bulk cereals was not affected appreciably by the storage temperature.

d. The retention of ascorbic acid in cereal discs varied inversely with the storage temperature.

e. A stabilized form of ascorbic acid in one of the disc experiments was no more stable than the regular form.

f. There was no advantage in using fortified gelatin or ground-up hard candy as a vehicle for ascorbic acid in cereals. These carriers apparently decreased the stability of ascorbic acid in premixed bulk cereals but had no effect on stability in the cereal discs.

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[3] Premixed cereals are mixtures of ready-to-eat cereal, sugar, dried, nonfat milk solids, and stable fat. These are packed in some rations as compressed discs or blocks and in others without compression (referred to in this report as "bulk"). Each manufacturer's product differs from others through being based on his commercial cereals.
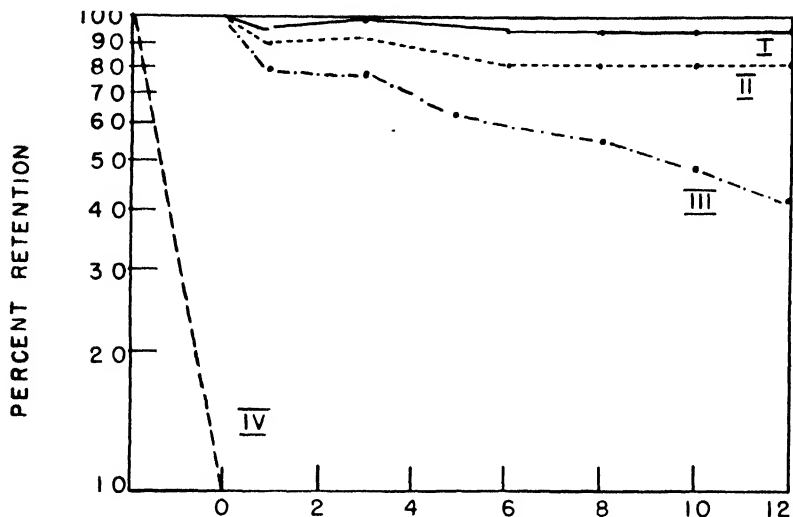
MONTHS STORAGE AT 100° F.

FIG. 4. Per cent retention of ascorbic acid in cereal and baked products on storage.

    I. Cookie filling.
   II. Bulk premixed cereal.
  III. Disc premixed cereal.
  IV. Fruit cake.

TABLE 3

Retention of Ascorbic Acid in Cereals as Affected by
Type of Carrier

Carrier	Bulk—6 months' storage			Disc—6 months' storage		
	Rm. temp.	100°F.	131°F.	Rm. temp.	100°F.	131°F.
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Manufacturer A						
Ascorbic acid......................	96	90	85	84	56	14
Ascorbic acid-hard candy..	....	77	....	77	64	20
Ascorbic acid gelatin.........	....	68	....	79	63	46
Stabilized ascorbic acid,						
Type 1......................	....	84	....	80	56	23
Stabilized ascorbic acid,						
Type 2......................	100	95	....	....	....	....
Manufacturer B						
Ascorbic acid, fine						
granulation....................	....	89	82	....	....	....
Ascorbic acid, coarse						
granulation....................	....	92	90	....	....	....
Stabilized ascorbic acid,						
Type 2......................	....	90	95	....	....	....
Manufacturer C						
Ascorbic acid......................	96	75	74	64[1]	62[1]	62[1]
Fortified gelatin...............	65	62	58	....	....	....
Average retention in						
cereals.....................	97	85	85	80	58	26

[1] Manufacturer C disc figures are for storage for four months and are not included in average.

g. The distribution of ascorbic acid in the cereals fortified with hard candy and gelatin was less uniform than in those fortified with added ascorbic acid as such.

h. Stability tests on fortified gelatin showed progressive losses up to three months' storage followed by a leveling-off of losses. Cereal discs showed the same type of losses.

The bulk cereal remained highly acceptable at all storage temperatures, whereas the disc darkened somewhat and had a slightly strong odor and flavor which were dissipated upon exposure to air.

A large-scale stability study on ration premixed bulk and disc cereal fortified with synthetic vitamins of the B-complex in addition to ascorbic acid, indicated approximately 75 per cent retention of the added ascorbic acid in the bulk and 65 per cent in the disc form. The decreased differences in stability of ascorbic acid in the bulk and disc forms in this set of cereals may be due to a stabilizing effect of one or all of the added B-complex vitamins.

Another type of carrier investigated in the cereal-and-baked-product category was sandwich cookie in which the filling, a product of hydrogenated fat, sugar, and flavoring, was fortified with approximately seven mg. of ascorbic acid per gram of filling. The cookie filling proved to be an excellent carrier for ascorbic acid as judged by the 95-per cent retention and good palatability at the end of six months' storage at 100°F. Other types of cookie fillings which were tested did not show as good stability. The value of the cookie filling as a carrier will depend upon the ability of the different producers to standardize the techniques used in making the filling so that good retentions of ascorbic acid will prevail as well as uniform distribution of filling from cookie to cookie. Such investigations are now in progress.

Early in the history of the laboratory the addition of ascorbic acid to baked products, such as fruit cake in loaf and cup-cake form, was also tried. Since the initial assays following the baking showed a 90-per cent destruction of ascorbic acid in these products, baked products were considered unsuitable as carriers. A combination cereal and fruit bar fortified with ascorbic acid suffered such great losses in flavor and ascorbic acid content in two months' storage that it, too, was discarded as a carrier. Stability studies were also carried out on plain fruit bars fortified with 40 mg. ascorbic acid per two-ounce bar or with an amount of guava to provide this quantity of ascorbic acid. Although there was slightly better stability in the ascorbic acid added as guava, both types of fruit bars lost over 90 per cent of the added ascorbic acid in three months' storage at 100°F.

Process American cheese and cheese spread with ham and relish were also investigated as possible carriers of ascorbic acid. Attainment of uniform distribution and good stability of the added vitamins was difficult, although the ascorbic acid was not discernible to the taste. Approximately 50 per cent of the added ascorbic acid was lost after three months' storage at 100° F. with an additional 30 per cent destruction after six months' storage at this temperature. Further work to increase the stability of ascorbic acid in cheese is planned.

DISCUSSION

Validity of Methods Used to Determine Ascorbic Acid Content of Stored Foods: Certain foods when stored at elevated temperatures for long periods of time develop interfering substances which compete with the ascorbic acid for reaction with the specific reagents used to determine the concentration of ascorbic acid, Levy (1943), Mapson (1943a, b), and Wokes, Organ, Duncan, and Jacoby (1943a, b). Such foods are usually high in carbohydrates, especially sugars, and the interfering substances are a result of caramelization of carbohydrates either alone or with protein at the high storage temperatures. Occasionally interfering materials may be present in foods prior to storage, as in the case of soluble coffee product which contains products of roasting similar to those which develop in other foods through storage. It is, therefore, necessary to modify the assay procedure where these substances occur so that the reaction measures only ascorbic acid rather than the total reaction owing to both ascorbic acid and biologically inactive products.

The validity of the data obtained on the stored food materials was checked by several means. One procedure was to assay the unfortified stored samples along with the fortified samples for the purpose of determining storage changes in the control. Several methods of assay employing different reactions were also used. These methods constituted not only the determination of the direct reduced and the direct dehydroascorbic acid as present in the food but also two measurements for total ascorbic acid, one made by converting all the ascorbic acid to the reduced form and the other by converting all the ascorbic acid to the dehydro form. It was hoped that the degree of agreement obtained for all four methods would aid in sifting out values which were due to materials other than ascorbic acid. In addition, the modified chemical methods for some materials were checked against biological assays. The results indicated that the chemical assays were measuring ascorbic acid activity (Table 4).

TABLE 4

Comparison of Chemical With Biological Values for Fortified Materials [1]

Product	G. P. assay	Roe	H_2S	Reduced
Soluble coffee product fortified with 25 mg. ascorbic acid (University of Wisconsin, Dr. Elvehjem)...........	25	......	......	25
Jam fortified with 30 mg. ascorbic acid (Nutrition Foundation, Dr. King)...............................	27.7	25.5	25.6	25.0
Jam fortified with guava and with 25 mg. ascorbic acid (Nutrition Foundation, Dr. King)......................	25.4	23.9	26.3	24.3
Premixed oat cereal fortified with 15 mg. ascorbic acid (Nutrition Foundation, Dr. King)......................	16.2	16.4	17.5	14.8
Premixed wheat cereal fortified with 15 mg. ascorbic acid (Nutrition Foundation, Dr. King)......................	17.5	14.9	18.0	15.9
Guava purée (American Can Co.. Dr. Pilcher)...............	200-250	220	241	213

[1] The biological values were furnished by institution and worker indicated under product.

Effect of Dehydroascorbic Acid Formation on Stability of Ascorbic Acid: Assays for dehydroascorbic acid content were made along with those on reduced ascorbic acid for the purpose of ascertaining whether there was a real loss in biological activity on storage. The results obtained on the products tested here, indicated that the reduction in the ascorbic acid content was due to a destructive loss of ascorbic acid rather than a conversion to the dehydro form (Fig. 5). It is clear that the loss of reduced ascorbic acid content on storage was not compensated by an increase in the dehydro form. The breakdown process in the ascorbic acid molecule, which was occurring on storage of fortified foods, did not stop at the dehydro stage long enough to be detected.
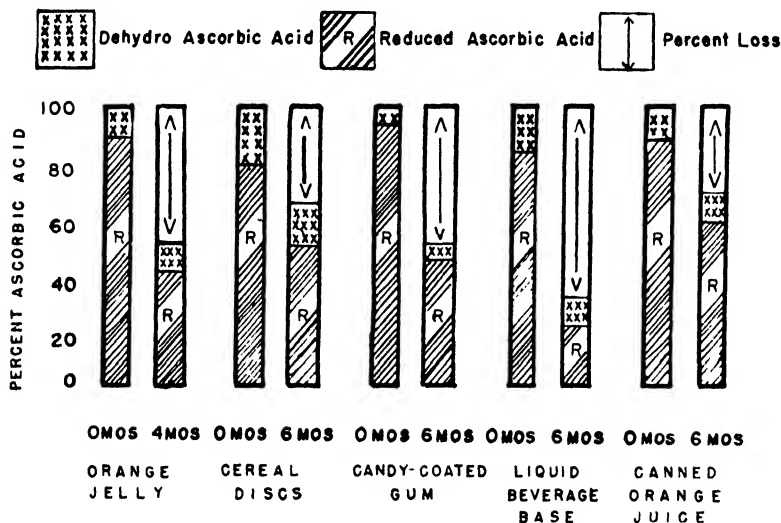
Fig. 5. Distribution of reduced and dehydroascorbic acid during storage at 100°F.

With the exception of cereal, the amount of dehydroascorbic acid found in fortified foods never exceeded one mg. per ounce initially or at any time during storage. In cereal, the initial amount ranged from one to three mg. per ounce and remained at that level throughout the storage period. Unfortified cereal also gave a positive reaction for the dehydroascorbic acid assay and the calculations were in the same range as in the fortified cereals, thus indicating that the substance measured was probably not dehydroascorbic acid.

Effect of Storage Temperature on Ascorbic Acid Stability: Although most of the products were stored only at room temperature and 100°F., several were stored at a rather complete range of temperatures, —3, 35, room temperature, 100, 110, 120, and 131°F. The use of the extreme high temperatures was contemplated as a rapid method for obtaining some indication about the stability of a carrier in a shorter period than was possible at the 100°F. storage temperature. It soon became apparent, however, that the validity of the data obtained at such temperatures was questionable inasmuch as new reactions were introduced and excessive destruction of the product occurred. Storage at temperatures beyond 100°F. was,

therefore, discontinued. Data at some of the above temperatures are available for the liquid orange-beverage base, for jams, for cereals, and for candy-coated gum. The stability of ascorbic acid in all these products was a function of the storage temperature and time as represented (Fig. 6).
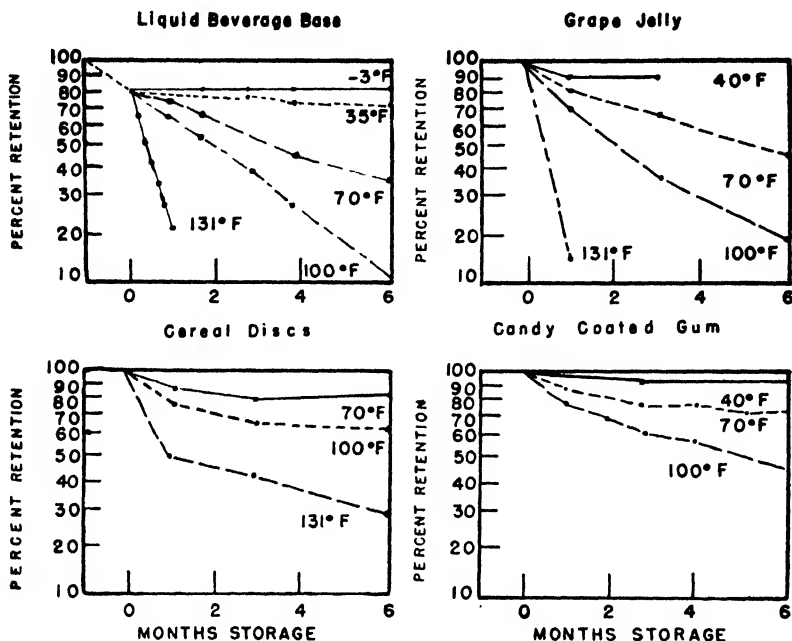

FIG. 6. Effect of storage temperature on ascorbic acid retention.

As the storage temperature and time increased, the losses of ascorbic acid also increased. This relationship was more spectacular in carriers with high moisture content than in those with low moisture content because of the increased rate of loss in the high moisture type of carriers. In some of the dry carriers (soluble coffee product, gelatin, etc.) the ascorbic acid losses at room temperature and 100°F. storage were approximately of the same magnitude. It is interesting to note that the products which were stable carriers of ascorbic acid at 131°F. storage temperature (hard candy and bulk cereal) showed no apparent deterioration to the point where they were unsatisfactory for consumption. This again demonstrates the relationship between ascorbic acid stability and palatability of the product. Such a correlation has been frequently obtained for citrus juices and other fresh fruits and vegetables.

"Stabilized" Ascorbic Acid: Several types of so-called "stabilized" ascorbic acid were investigated along with the regular-type ascorbic acid in various products. In essence the stabilized ascorbic acid is ordinary ascorbic acid with some type of protective coating around the crystal. The coating acts as a protective agent against moisture and oxygen, and once it is removed by heat or solution the stabilized ascorbic acid becomes the ordinary crystal again and is liable to moisture, oxygen, and heat as if it

had never been protected. The application of stabilized ascorbic acid to improve the stability of carriers is, therefore, limited. It could not be used in materials to which it is added while the carrier is hot, as is the case with many of the soft confections, jams, and jellies. The stabilized form may have possibilities in the cereal discs, sugar, and other dry carriers where no wet mixing or high temperatures are necessary.

A comparison of the stability of regular and stabilized ascorbic acid in several carriers (Table 5) shows that the use of the stabilized form of ascorbic acid did not result in preventing losses.

TABLE 5

Retention of Ascorbic Acid in Carriers Fortified With Stabilized and Ordinary Ascorbic Acid

Carrier	Ordinary ascorbic acid, storage at 100°F.			Stabilized ascorbic acid, storage at 100°F.		
	1 mo.	3 mo.	6 mo.	1 mo.	3 mo.	6 mo.
Soluble coffee product............................	....	93	90	95	83	80
Cocoa beverage powder						
Bulk...	100	93	96	93	90	92
Disc...	....	85	68	....	85	65
Premixed cereal disc.............................	66	60	55	64	60	55
Starch-jelly confection.........................	75	38	....	93	70	60

SUMMARY

Of the carriers tested thus far, the following were judged to be stable carriers based on the criterion of a minimum retention of 75 to 80 per cent of the added ascorbic acid after six months' storage at 100°F.:

(a) Synthetic fruit-juice powders.

(b) Hard-pressed mints, candy wafers, and coated jelly beans.

(c) Bulk cocoa beverage powder and soluble coffee product.

(d) All types of premixed bulk cereal.

(e) Some types of filling used in sandwich cookies.

(f) Bulk and disc granulated sugar.

The following were found to be borderline carriers (60 per cent retention of the added ascorbic acid after six months' storage at 100°F.):

(a) Cereal discs and cocoa beverage-powder discs.

(b) Gelatin.

(c) Dehydrated orange juice and canned orange juice.

The following were found to be unsatisfactory carriers of ascorbic acid (0 to 45 per cent retention of the added ascorbic acid after six months' storage at 100° F.):

(a) Some types of dehydrated orange-juice powders and liquid bases.

(b) Chocolate, caramel, lozenges, and starch-jelly confection.

(c) Jams and jellies with or without guava.

(d) Gum, stick and candy-coated, ascorbic acid in gum center or in candy coating.

(e) Fruit cake and cereal fruit bar.

(f) Fruit bar fortified with ascorbic acid or guava.

No appreciable losses in the ascorbic acid content of beverage powders resulted from the preparation of either a hot or cold beverage.

The assay methods used in the determination of ascorbic acid in stored foods were valid as indicated by comparisons with biological assays.

The ascorbic acid losses sustained during storage were true losses and not changes to dehydroascorbic acid.

The quantities of dehydroascorbic acid present initially in the fortified foods studied were insignificant except in premixed cereals where the apparent amount was about 16 to 20 per cent. The same amount of "dehydro" ascorbic acid was found in samples of unfortified cereals. It is, therefore, believed that the positive reaction is due to interfering substances present in premixed cereals.

Losses in ascorbic acid content were in general related to the manufacturing process, storage time, storage temperature, moisture content, and acidity of the carrier as well as the type of structure into which the ascorbic acid was incorporated. The more impenetrable the internal and external surfaces of the carrier to air and moisture, the more stable was the carrier.

All stable carriers tested (premixed bulk cereal, hard candy, synthetic beverages, and sandwich cookies) retained satisfactory palatability. Not all poor carriers, however, (chocolate, caramel, starch-jelly confection, etc.) lost their acceptability.

The use of "stabilized" ascorbic acid in several carriers did not improve the stability sufficiently.

Uniformity of distribution of ascorbic acid from unit to unit was poor in many items, especially in premixed cereals containing large granules.

There was no advantage to adding ascorbic acid to carriers in the form of other normally stable carriers, such as ground-up hard candy. This increased the distribution difficulties without increasing stability.

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COMPOSITION, INCLUDING THIAMINE AND RIBOFLAVIN, OF EDIBLE DRY LEGUMES [1]

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There are practically no data available in the literature as to the chemical composition of the most widely used edible dry legumes. Their use as foods has increased during the past few years and greater consumption is being urged by some food specialists. Large quantities of low-grade lots and culls are also being used for animal feed, a field in which the composition is of equal importance. Recently, Daniel and Norris (1945) published thiamine, niacin, and riboflavin analyses of several samples of dried legumes. They also reviewed the literature of some of the early vitamin work.

Forty samples of legumes were obtained for this study. They were grown in the two major legume-producing areas in Idaho, in the north around Moscow and in the south in the vicinity of Twin Falls. Only six samples were from the 1944 crop and all others from the 1945 crop.

EXPERIMENTAL PROCEDURE

The composition as to moisture, crude protein, crude fat, crude fiber, nitrogen-free extract, and ash were determined by the official A.O.A.C. methods (1940) and phosphorus by the method of Bolin and Stamberg (1944). Riboflavin and thiamine were determined essentially by the photofluorometric methods of Conner and Straub (1941). The types of peas, beans, and lentils used are shown (Table 1).

DISCUSSION AND SUMMARY

The data (Table 1) show that there is some variation in protein content of the various legumes, the lentils being highest with an average content of 29.2 per cent and the small red beans lowest at 21.8 per cent. All of the legumes were fairly high in phosphorus. The data on riboflavin and thiamine agree fairly well with most of the values presented by Daniel and Norris, except for the thiamine content of the lentils which is slightly less than half their value. All samples were quite low in crude fat and averaged only slightly over one per cent. The high protein, phosphorus, thiamine, and relatively high riboflavin content of legumes should make them valuable as a food and for livestock feeding if properly balanced with other nutrients.

ACKNOWLEDGMENT

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TABLE 1

Results of Analyses of Edible Dry Legumes

Variety	Mois-ture	Crude protein	Crude fat	Crude fiber	Nitro-gen-free extract	Ash	Phos-phorus	Ribo-flavin	Thiamine
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*µg./gm.*	*µg./gm.*
Alaska field peas (green)	6.55	26.0	1.30	3.68	59.78	2.69	.408	3.25	6.84
	8.50	25.5	1.16	3.07	58.95	2.82	.360	2.91	7.44
	7.54	24.7	1.25	4.04	59.82	2.65	.418	2.99	7.16
	5.58	26.0	1.14	2.96	61.65	2.67	.348	2.97	6.96
Average........	7.04	25.6	1.21	3.44	60.05	2.71	.384	3.03	7.10
First and best peas (yellow)	8.51	27.6	1.01	4.09	56.35	2.44	.386	3.69	7.76
	8.01	29.3	1.24	4.25	54.99	2.21	.414	3.11	8.96
	9.07	27.7	1.26	4.65	55.04	2.28	.414	3.23	8.36
	9.13	26.4	1.21	4.67	56.39	2.20	.450	3.19	7.20
Average........	8.68	27.8	1.18	4.42	55.69	2.28	.416	3.31	8.07
Flat small white beans	8.46[1]	22.5	1.39	6.14	56.91	4.60	.450	3.07	5.76
	9.89	22.4	1.20	4.78	57.31	4.42	.466	2.96	8.64
	8.18	22.3	1.38	5.03	58.79	4.32	.492	2.66	9.08
Average........	8.84	22.4	1.32	5.32	57.67	4.45	.469	2.90	7.83
Small red beans	7.57[1]	22.8	1.42	4.11	60.14	3.96	.492	2.72	7.48
	8.73	20.9	1.20	3.10	62.13	3.94	.434	2.66	6.20
	7.30	20.8	1.65	3.83	62.47	3.95	.454	2.44	7.00
	8.09	21.5	1.53	3.82	61.28	3.78	.450	2.51	6.84
	7.76	20.8	1.24	3.69	62.58	3.93	.452	2.31	6.44
	8.75	22.4	1.38	3.79	59.74	3.94	.394	2.82	6.24
	7.16	22.4	1.20	3.77	61.52	3.95	.388	2.54	6.80
	7.85	22.6	1.45	4.29	60.01	3.80	.492	2.63	6.92
	5.53	22.4	1.47	8.83	57.83	3.94	.372	2.43	6.92
Average........	7.64	21.8	1.39	4.36	60.86	3.91	.436	2.56	6.76
Pinto beans	8.66[1]	23.3	1.35	4.31	58.26	4.12	.484	3.10	5.72
	7.64	22.9	1.37	4.65	59.78	3.66	.454	2.52	5.44
	7.80	23.4	1.16	4.78	58.66	4.20	.414	2.79	5.16
	7.64	23.3	1.45	4.78	58.72	4.11	.348	2.55	5.16
	8.74	23.7	1.35	4.62	57.49	4.10	.376	2.59	4.96
Average........	8.09	23.3	1.34	4.63	58.58	4.04	.415	2.71	5.29
Western red kidney beans	7.54[1]	23.9	1.31	3.84	59.45	3.96	.400	2.99	5.56
	7.72	22.2	1.16	3.84	61.59	3.49	.350	2.57	5.92
Average........	7.63	23.1	1.24	3.84	60.52	3.73	.375	2.78	5.74
Great Northern beans	8.70[1]	24.7	1.05	3.67	57.56	4.32	.520	3.42	7.92
	8.34	24.4	1.30	3.32	58.92	3.72	.512	3.17	8.56
	8.32	24.5	1.14	3.82	57.71	4.51	.506	2.76	8.04
	7.31	23.8	1.20	3.67	60.35	3.67	.436	2.75	7.40
	8.16	23.9	1.22	3.68	58.49	4.55	.454	3.00	7.56
	5.51	24.1	1.38	3.81	60.73	4.47	.340	3.02	7.36
	7.36	24.0	1.19	3.84	59.60	4.01	.388	2.99	9.82
	6.56	24.0	1.31	3.68	60.70	3.75	.440	2.90	7.72
Average........	7.53	24.2	1.22	3.69	59.26	4.13	.450	3.00	8.05
Pea beans	8.53[1]	23.1	1.22	4.65	58.42	4.08	.492	2.90	7.32
	8.11	23.1	1.87	4.65	58.50	3.77	.400	2.86	7.24
Average........	8.32	23.1	1.55	4.65	58.46	3.93	.446	2.88	7.28
Lentils	7.74	29.1	0.89	3.86	54.64	3.77	.492	3.01	2.96
	8.74	29.2	0.94	4.64	52.50	3.98	.498	2.90	2.80
	7.62	29.2	0.85	5.04	53.34	3.95	.492	2.94	2.68
Average........	8.03	29.2	0.89	4.51	53.49	3.90	.494	2.95	2.81

[1] From 1944 crop, all others 1945 crop.

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NUTRITIVE VALUE OF CANNED FOODS [1]

XXI. RETENTION OF NUTRIENTS DURING COMMERCIAL PRODUCTION OF VARIOUS CANNED FRUITS AND VEGETABLES

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The urgent need for more information on the nutritive value of canned foods has led to an extensive series of investigations sponsored jointly by the National Canners Association and the Can Manufacturers Institute, Clifcorn (1944). One phase of these investigations has been an attempt to improve the nutritional value of canned food by the elimination or modification of those canning practices found to be destructive of nutrients and to promote the development of canning procedures giving the maximum retention of the nutrients present in the raw product.

Wagner, Strong, and Elvehjem (1947) have studied the effect of commercial canning operations on the ascorbic acid, thiamin, riboflavin, and niacin content of vegetables and have presented an extensive review of the literature on this subject.

Guerrant, Vavich, Fardig, Dutcher, and Stern (1946) have conducted similar studies to determine some of the factors or conditions associated with canning procedures which are conducive to high vitamin retention. The investigation involved nine vegetables and one fruit and the canning procedures of 27 different establishments.

The purpose of the investigation reported in this paper was to obtain further information on the retention of the water-soluble vitamins and carotene during the commercial canning of apricots, asparagus, green beans, carrots, corn, peaches, peas, spinach, tomatoes, and tomato juice. Studies were conducted on the above products during the 1943, 1944, and 1945 canning seasons in canneries located in California and in the Pacific Northwest. Data obtained by this laboratory on the retention of ascorbic acid in grapefruit juice and in orange juice are given by Lamb (1946).

METHODS

Ascorbic Acid: Ascorbic acid was determined according to a modification of the method of Bessey and King (1933) for all products except asparagus.

Slurries were prepared by mixing from 200 to 300 grams of material with an equal weight of extracting solution in a Waring blendor. For tomatoes, tomato juice, and asparagus the extracting solution contained three per cent metaphosphoric acid; for peas, spinach, and peaches, six

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per cent metaphosphoric acid; and for apricots, green beans, and corn, a mixture of six per cent metaphosphoric acid and one per cent oxalic acid. Peas, corn, and a portion of the green bean samples were blended in an atmosphere of nitrogen; all others were blended in air. All products except corn were blended for two minutes; corn was blended for five minutes. The stability of ascorbic acid in each product during extraction was tested prior to making actual retention experiments in order that the most suitable extraction procedure would be used.

After blending, 20 to 50 grams of the resulting slurry were transferred to a 100-ml. volumetric flask and made up to volume with three per cent metaphosphoric acid solution. The contents of the flask were mixed and filtered, and a 10- or 20-ml. aliquot titrated with 2,6-dichlorobenzenoneindophenol solution (50 mg. + 20 mg. sodium bicarbonate per 100 ml. of solution).

Since difficulty was experienced in filtering corn extracts, the flasks were allowed to stand until the solids had settled, and then an aliquot of the supernatant liquid was removed for titration.

Because of the presence of interfering red pigments in raw asparagus and the tendency for the end point to fade rapidly, asparagus could not be titrated successfully with the indophenol dye. Ascorbic acid was determined in this product by a modification of the photometric method of Loeffler and Ponting (1942). Twenty grams of the blended extract were diluted to 100 ml. with 0.9 per cent metaphosphoric acid solution (to give a final concentration of one per cent metaphosphoric acid), and the determination was completed according to the Loeffler and Ponting method using either a Cenco photelometer or an Evelyn colorimeter.

B Vitamins: Three hundred grams of material were blended with an equal weight of 0.1 N sulfuric acid until a homogeneous slurry was obtained. During the blending two ml. of chloroform were added. The contents were then transferred to a pint glass jar and two ml. of toluene layered on the surface of the sample. The jars were packed in a shipping container with dry ice and shipped to the San Francisco laboratory for analysis. After arrival at the laboratory, the samples were held at 1.1°C. (34°F.) until the determinations of thiamin, riboflavin, and niacin could be completed.

Thiamin was determined by the thiochrome method of Hennessy (1941) with the following modifications:

1. Samples of green beans were extracted for two hours with 0.1 N H_2SO_4 in a boiling-water bath; all other samples were extracted for one hour.

2. Samples were incubated overnight at 37°C.(98.6°F.) with one gram of takadiastase.

3. The decalso was activated by stirring for 15 minutes with hot three-per cent acetic acid. The acetic acid was decanted and the decalso stored under three-per cent acetic acid.

4. Thiamin was eluted from the decalso by passing 50 ml. of a hot 25-per cent sodium chloride solution through the adsorption column.

Riboflavin was determined by the microbiological method of Snell and Strong (1939) and niacin by the microbiological method of Snell and Wright (1941) as modified by Krehl, Strong, and Elvehjem (1943).

Carotene: Three hundred grams of material were blended with an equal weight of 95 per cent alcohol containing either 1.0 or 1.5 per cent of potassium hydroxide. The higher concentration was used for apricots, the lower concentration for peas and carrots. The blended material was transferred to a glass jar and packed in a shipping container with dry ice, in which it was transported to the San Francisco laboratory for analysis. After arrival at the laboratory the samples were held at 34°F. until they could be tested.

The samples were analyzed according to the extraction procedure of Moore and Ely (1941) and the chromatographic procedure of Wall and Kelly (1943). The carotene solutions were read either in a Cenco photelometer or an Evelyn colorimeter calibrated against a purified solution containing a mixture of 90 per cent beta- and 10 per cent alpha-carotene.

Moisture: Moisture was determined on the blended sample by the official A.O.A.C. method for tomato products (1940). The sample for moisture determination was prepared by adding an accurately weighed portion of the material to a No. 2 or a No. 2½ can. The can was filled with hot distilled water and then closed and processed in the regular manner. Upon opening, the net weight of contents of the can was determined, and the ratio of net weight to initial weight of product was applied to the value obtained for moisture content.

EXPERIMENTAL PROCEDURE

At each of the canneries samples were taken of the raw product, of the same lot of raw material after it had undergone various treatments preparatory to canning, and finally of the finished product, by one of the following procedures:

Batch Operation: If the canning was a batch operation, material was sampled continuously from each sampling point while a particular batch was being run. If thorough mixing of the material in the batch was assured, the sample material was taken at one time, after about half the batch was run through the equipment.

Continuous Operation: If the canning was a continuous operation, each operation was first timed with a stop watch by following a marker placed in the line, and the time required for the product to travel from one sampling point to another was recorded. Samples were then taken from each sampling point over a predetermined period of time, the sampling times being so scheduled as to represent the same original material at each sampling point. Wherever possible, markers were placed in the line at the beginning and end of the sampling period in order to insure selection of the same material at each sampling point. Canning operations were continuous in most of the canneries included in these studies.

The unstable nature of the material sampled at certain places in the canning line made it imperative that sampling be completed in as brief a period of time as possible; on the other hand, in order to obtain representative samples from a sufficiently large amount of material a certain

minimum time was required. Sampling periods varied from two to 10 minutes, but ordinarily the time selected was not over five minutes. The material was sampled at a uniform rate during the sampling period, at the end of which time from five to 10 pounds of material had been accumulated. From 16 to 18 cans were selected during the predetermined period and, after coding, 12 of the cans were returned to the canning line and given the regular processing treatment. The remaining cans were cooled to room temperature by immersion in running water, after which they were opened and the material prepared for analysis.

The material from each sampling point was well mixed and two or three samples consisting of from 200 to 300 grams of material were quickly weighed out for ascorbic acid determinations and mixed with an equal weight of stabilizing solution. Following this, a 300-gram sample was weighed out for determination of B vitamins and preserved in the manner previously described. A sample for carotene was similarly prepared in those instances where this vitamin was to be determined. The analyses for ascorbic acid were performed on the spot, usually within an hour after the samples were collected.

Wherever possible, the history of the raw product was determined and samples were selected from material grown in a single field. In certain of the larger canneries it proved impossible to trace the exact history of the raw product after it reached the cannery. In several instances it had gone through a receiving station previous to shipment to the cannery and had lost its identity or it had become mixed after being received at the cannery. Very often the canning operations necessitated a blending of the raw product from several growers.

In all instances, considerable care was taken not to interfere with canning operations in use at the cannery being surveyed at the time the tests were made. All retention values, therefore, represent actual commercial conditions of canning and were not influenced by the manner in which samples were selected.

In order to obtain the necessary laboratory facilities for carrying on this work in canneries, a mobile laboratory which could be moved from one cannery to another was constructed. This unit could be set up inside a cannery and could be ready for operation as soon as electrical and water connections were made to cannery sources.

RETENTION OF ASCORBIC ACID, THIAMIN, RIBOFLAVIN, NIACIN, AND CAROTENE DURING CANNING PROCEDURE

A summary of vitamin retention values in apricots, green asparagus, white asparagus, green beans, carrots, corn, clingstone peaches, freestone peaches, peas, and tomatoes is presented (Table 1); a summary of the actual vitamin values obtained on the raw and processed samples is also included. Only the vitamins present in sufficient concentration in the raw product to be significant from a nutritional standpoint are included.

Maximum, minimum, and average retentions are included (Table 1) and standard deviation in cases where a sufficient number of individual values were obtained to warrant such treatment of the data. In calculation of standard deviation the assumption was made that the values show ran-

TABLE 1

Vitamin Retention During Commercial Production of Various Canned Fruits and Vegetables

Sample	Vitamin	Number of retention experiments	Actual values			Retention (dry basis)		
			Max.	Min.	Av.	Max.	Min.	Av. & S.D.[1]
			mg./ 100 gm.	mg./ 100 gm.	mg./ 100 gm.	pct.	pct.	pct.
APRICOTS (3 canneries)								
Raw	Ascorbic acid	9	12.60	8.20	10.20	......	......	100
Processed	Ascorbic acid	9	7.40	4.30	5.60	97	76	87±7
Raw	Carotene	9	2.14	0.81	1.59	......	......	......
Processed	Carotene	9	1.24	0.49	0.88	98	78	89±8
ASPARAGUS, GREEN (5 canneries)								
Raw	Ascorbic acid	19	50.20	17.30	30.60	......	......	100
Blanched	Ascorbic acid	16	......	......	......	117	90	100±7
Exhausted	Ascorbic acid	13	......	......	......	116	82	101±10
Processed	Ascorbic acid	19	30.70	11.10	19.40	116	90	100±7
Raw	Thiamin	11	0.24	0.15	0.18	......	......	100
Blanched	Thiamin	5	......	......	......	104	83	91±8
Processed	Thiamin	11	0.09	0.06	0.08	78	51	65±7
Raw	Riboflavin	12	0.23	0.15	0.19	......	......	100
Blanched	Riboflavin	5	......	......	......	103	74	92±10
Processed	Riboflavin	12	0.13	0.10	0.11	109	72	88±11
Raw	Niacin	11	1.80	1.40	1.59	......	......	100
Blanched	Niacin	3	......	......	......	108	87	96±7
Processed	Niacin	11	1.05	0.86	0.94	105	76	93±7
ASPARAGUS, WHITE (6 canneries)								
Raw	Ascorbic acid	11	33.40	17.50	27.90	......	...... ⋅	100
Blanched	Ascorbic acid	10	......	......	......	118	74	96±11
Exhausted	Ascorbic acid	8	......	......	......	105	91	100±6
Processed	Ascorbic acid	11	22.70	11.10	18.10	109	85	96±8
Raw	Thiamin	10	0.15	0.12	0.11	......	......	100
Blanched	Thiamin	5	......	......	......	102	79	91±9
Processed	Thiamin	10	0.08	0.05	0.06	87	62	73±7
Raw	Riboflavin	10	0.15	0.11	0.13	......	......	100
Blanched	Riboflavin	5	......	......	......	103	72	92±13
Processed	Riboflavin	10	0.09	0.06	0.08	99	65	85±13
Raw	Niacin	10	1.29	0.98	1.16	......	......	100
Blanched	Niacin	5	......	......	......	105	77	95±11
Processed	Niacin	10	0.91	0.67	0.81	111	94	102±6
BEANS, GREEN (5 canneries)								
Raw	Ascorbic acid	25	18.80	5.60	9.40	......	......	100
Blanched	Ascorbic acid	25	......	......	......	96	48	74±13
Closed	Ascorbic acid	25	......	......	......	86	29	60±15
Processed	Ascorbic acid	25	6.80	1.40	3.40	81	35	60±9
Raw	Thiamin	24	0.12	0.07	0.09	......	......	100
Blanched	Thiamin	23	......	......	......	108	75	94±9
Closed	Thiamin	22	......	......	......	113	79	98±10
Processed	Thiamin	24	0.07	0.04	0.05	88	57	73±9
Raw	Riboflavin	24	0.17	0.09	0.10	......	......	100
Blanched	Riboflavin	23	......	......	......	102	87	97±4
Closed	Riboflavin	23	......	......	......	110	86	99±6
Processed	Riboflavin	24	0.09	0.05	0.06	106	85	97±6
Raw	Niacin	24	0.74	0.37	0.45	......	......	100
Blanched	Niacin	23	......	......	......	113	79	97±6

TABLE 1 (*Continued*)

*Vitamin Retention During Commercial Production of Various
Canned Fruits and Vegetables*

Sample	Vitamin	Number of retention experiments	Actual values			Retention (dry basis)		
			Max.	Min.	Av.	Max.	Min.	Av. & S.D.[1]
			mg./ 100 gm.	mg./ 100 gm.	mg./ 100 gm.	pct.	pct.	pct.
Closed......................	Niacin	22	......	......	......	110	86	99±7
Processed..................	Niacin	24	0.45	0.15	0.25	105	81	95±6
CARROTS								
(1 cannery)								
Raw..........................	Carotene	5	14.80	10.20	12.10	......	......	100
After dicing.............	Carotene	5	......	......	......	106	97	101±3
After steaming.........	Carotene	5	......	......	......	115	108	112±3
Processed..................	Carotene	5	6.30	9.40	7.40	113	97	106±6
CORN								
(1 cannery)								
Raw..........................	Ascorbic acid	3	13.70	11.60	12.60	......	......	100
Cut and washed........	Ascorbic acid	3	......	......	......	88	84	85
Exhausted.................	Ascorbic acid	3	......	......	......	83	71	76
Processed..................	Ascorbic acid	3	7.80	6.00	6.80	94	75	85
Raw..........................	Thiamin	3	0.16	0.16	0.16	......	......	100
Cut and washed........	Thiamin	3	......	......	......	86	68	79
Exhausted.................	Thiamin	3	......	......	......	93	81	86
Processed..................	Thiamin	3	0.10	0.09	0.09	43	28	35
Raw..........................	Riboflavin	3	0.12	0.11	0.11	......	......	100
Cut and washed........	Riboflavin	3	......	......	......	100	98	99
Exhausted.................	Riboflavin	3	......	......	......	105	96	101
Processed..................	Riboflavin	3	0.07	0.08	0.07	105	99	102
Raw..........................	Niacin	3	2.25	1.97	2.13	......	......	100
Cut and washed........	Niacin	3	......	......	......	92	88	90
Exhausted.................	Niacin	3	......	......	......	87	81	83
Processed..................	Niacin	3	1.13	1.04	1.08	86	77	82
PEACHES,								
CLINGSTONE								
(4 canneries)								
Raw..........................	Ascorbic acid	14	15.50	8.60	10.90	......	......	100
Lye-peeled................	Ascorbic acid	14	......	......	......	91	61	82±9
Exhausted.................	Ascorbic acid	14	......	......	......	81	47	63±11
Processed..................	Ascorbic acid	14	6.60	4.10	5.10	90	63	71±8
Raw..........................	Niacin	5	1.24	0.88	1.01	......	......	100
Lye-peeled................	Niacin	5	......	......	......	99	95	97±2
Exhausted.................	Niacin	5	......	......	......	93	84	90±2
Processed..................	Niacin	5	0.72	0.54	0.62	92	87	89±1
PEACHES,								
FREESTONE								
(1 cannery)								
Raw..........................	Ascorbic acid	3	8.00	6.30	7.00	......	......	100
Steam-peeled............	Ascorbic acid	3	......	......	......	86	72	77
Processed..................	Ascorbic acid	3	3.90	2.20	2.90	70	59	65
Raw..........................	Niacin	3	0.98	0.89	0.93	......	......	100
Processed..................	Niacin	3	0.57	0.51	0.53	86	82	84
PEAS								
(5 canneries)								
Raw..........................	Ascorbic acid	26	32.60	20.80	27.00	......	......	100
Blanched...................	Ascorbic acid	26	......	......	......	88	57[a]	78±6
Closed.......................	Ascorbic acid	24	......	......	......	87	43[a]	78±7
Processed..................	Ascorbic acid	26	15.40	8.30	11.70	88	43[a]	74±7

TABLE 1 (*Concluded*)

Vitamin Retention During Commercial Production of Various
Canned Fruits and Vegetables

Sample	Vitamin	Number of retention experiments	Actual values			Retention (dry basis)		
			Max.	Min.	Av.	Max.	Min.	Av. & S.D.[1]
			mg./ 100 gm.	mg./ 100 gm.	mg./ 100 gm.	pct.	pct.	pct.
Raw...........................	Carotene	6	0.46	0.33	0.39	......	......	100
Processed..................	Carotene	6	0.29	0.24	0.26	127	106	118±8
Raw...........................	Thiamin	24	0.40	0.20	0.29	......	......	100
Blanched...................	Thiamin	24	......	......	......	108	70	90±8
Closed.......................	Thiamin	21	......	......	......	116	70	92±14
Processed..................	Thiamin	24	0.14	0.05	0.09	83	42	57±9
Raw...........................	Riboflavin	26	0.21	0.14	0.17	......	......	100
Blanched...................	Riboflavin	26	......	......	......	89	61	76±6
Closed.......................	Riboflavin	14	......	......	......	96	70	82±7
Processed..................	Riboflavin	26	0.10	0.06	0.08	96	67	82±7
Raw...........................	Niacin	26	4.19	2.06	3.23	......	......	100
Blanched...................	Niacin	26	......	......	......	94	50	71±10
Closed.......................	Niacin	13	......	......	......	76	55	66±6
Processed..................	Niacin	26	2.03	0.68	1.29	85	53	67±8
TOMATOES (3 canneries)								
Raw...........................	Ascorbic acid	5	27.50	20.20	23.60	......	......	100
Processed..................	Ascorbic acid	5	24.10	17.30	20.30	91	86	88

[1] S. D. = Standard deviation. [2] These values were obtained on No. 2 sieve size peas held for a considerable length of time between blanching and processing and were excluded from the average and standard deviation. The next lowest retentions were blanched, 64 per cent; closed, 60 per cent; processed, 56 per cent.

dom distribution about a mean and are not unduly influenced by the choice of variables selected. Although the above assumption may not be justified in every case, it is felt that the inclusion of standard deviation is an aid in interpretation of the data in that it provides an estimate of the most probable range of retentions which would be obtained for a given vitamin in a given product. The maximum and minimum values are often misleading in that they may be the result of abnormal circumstances and not a true indication of actual commercial conditions.

Individual retention values in excess of 100 per cent are probably due to a preponderance of positive errors in those particular experiments. It may be assumed that the average of a random distribution of retention values is equally affected by positive and negative errors and, therefore, is more significant than any individual retention value. An average retention appreciably over 100 per cent probably indicates a consistent positive error in a particular vitamin determination. That this is true in the case of carotene retention will be discussed later.

The original data do not reveal significant differences between different canneries packing the products shown (Table 1). In most instances variations between separate retention experiments performed in the same cannery were as great as variations in retention experiments performed in different canneries. Although canning procedure differed considerably between canneries packing the same product, these differences appeared to be less important than variations in the characteristics of the raw

product, such as variety, sieve size, maturity, etc. Differences in retention between individual canning procedures might have become more apparent had it been possible to perform retention experiments on identical lots of raw material and to limit variations in canning procedure to a single variable, such as blanching, exhausting, etc.

Retention of Ascorbic Acid in Spinach: In contrast to the products shown (Table 1), distinct differences were found in the retention of ascorbic acid obtained at different spinach canneries. A summary of actual values and retention values for ascorbic acid obtained as a result of surveys performed at five spinach canneries is shown (Table 2). The results obtained during separate runs performed at the same cannery agreed fairly well but differed markedly in several instances from runs made in other canneries. Variations in blanching procedures appear to account for the major differences in retention of ascorbic acid in spinach. Additional data obtained by this laboratory on the effect of blanching on the retention of nutrients in spinach indicate that the length of blanch and the type of blanching equipment have a pronounced effect on the retention of water-soluble nutrients.

TABLE 2

Retention of Ascorbic Acid in Canned Spinach

Cannery No.	Number of retention experiments	Average ascorbic acid content		Average retention of ascorbic acid (dry basis)			
		Raw	After processing	After washing	After blanching	After exhausting	After processing
		mg./100 gm.	mg./100 gm.	pct.	pct.	pct.	pct.
1	5	85	20.0	......	49	42	50
2	6	73	20.6	117	77	59	62
3	4	87	12.9	99	39	27	34
4	6	74	23.7	108	78	56	. 60
5	4	83	4.8	63[1]	8	1	14

[1] Warm water wash (160°F.).

Retention of Ascorbic Acid, Thiamin, Riboflavin, and Niacin in Tomato Juice: The retention of ascorbic acid in tomato juice also varied considerably from one cannery to another. Since the data obtained on this product are difficult to summarize, owing to the great variability of canning technics, a few individual retention experiments were selected for presentation (Table 3). Each experiment represents a different canning technic and was chosen from a large mass of similar data as being representative of the various canning technics being employed in California canneries.

In Cannery 1 the tomatoes were not heated prior to juice extraction. In Cannery 2 the tomatoes were steamed, crushed, and then heated to 85°C. (185°F.) prior to juice extraction. In Canneries 3 and 4 the tomatoes were steamed prior to crushing but were not given further heating prior to juice extraction. In Cannery 5 the tomatoes were heated to 87.8°C. (190°F.) at the time of crushing. Canneries 1 and 4 employed deaeration, whereas the·other canneries did not. At Cannery 5 the juice was held about 45 minutes prior to canning. At the other canneries the time of holding varied from approximately three to 20 minutes.

In Canneries 2, 3, and 5 the juice was preheated to 82.2 to 93.3°C.(180 to 200°F.), filled into cans, and processed either in continuous cookers or in retorts. In Canneries 1 and 4 the juice was rapidly heated to 110 to 121.1°C.(230 to 250°F.), after which it was cooled to about 200°F., filled, and air-cooled without further processing.

TABLE 3

Vitamin Retentions in Canned Tomato Juice

Cannery No.	Sample	Ascorbic acid	Thiamin	Riboflavin	Niacin	Retention of ascorbic acid
		mg./100 ml.	mg./100 ml.	mg./100 ml.	mg./100 ml.	pct.
1	Raw, juiced...........................	27.2	.057	.024	.77	......
	From finisher.........................	28.0	......	......	......	100
	After closing.........................	26.2	.062	.024	.86	94
	After cooling.........................	26.1	.062	.025	.88	93
2	Raw, crushed.........................	28.1	.058	.030	.92	100
	After preheating.....................	27.2	......	......	......	97
	From finishing tank...............	23.0	......	......	......	82
	From filler............................	21.6	.056	.028	.80	77
	Processed............................	21.5	.056	.027	.76	77
3	Raw, juiced...........................	24.8	.041	.024	.60	......
	Steamed, juiced.....................	28.2	......	......	......	100
	After cycloning.....................	25.2	......	......	......	89
	From finisher.........................	24.8	......	......	......	88
	From holding tank.................	15.3	......	......	......	54
	From filler............................	15.1	.041	.023	.58	54
	Processed............................	15.4	.040	.023	.59	55
4	Raw, juiced...........................	26.5	......	......	......	......
	Steamed and crushed............	30.0	......	......	......	100
	From finisher.........................	27.8	......	......	......	93
	From filler............................	28.4	......	......	......	95
	Closed and cooled.................	28.2	......	......	......	94
5	Raw, steamed.........................	27.5	......	......	......	100
	Chopped, heated to 190°F...	19.7	......	......	......	72
	From finisher.........................	12.4	......	......	......	45
	From filler............................	8.3	......	......	......	30
	Processed............................	8.8	......	......	......	32

The data show that whereas it is possible to obtain excellent retention of ascorbic acid in tomato juice, there is considerable variation in the retentions actually obtained. The use of deaerators appears to be of particular advantage in obtaining high retention of ascorbic acid.

The over-all retentions obtained at the different tomato-juice canneries are not exactly comparable since the value obtained on the "raw" sample depended to a certain extent upon whether or not the tomatoes were steamed prior to juice extraction. A home-type juice extractor was used in extracting the juice representing the "raw" and "steamed" samples in all cases. In every case steaming has served to produce a juice richer in ascorbic acid than extraction of the cold tomatoes. This factor is responsible for the observation that in Cannery 4 the processed juice appears to

contain more ascorbic acid than the original raw juice. Possible explanations for the apparent increase in ascorbic acid after steaming the tomatoes are either that the steaming serves to inactivate certain enzymes beneath the skin which promote the oxidation of ascorbic acid, or that the steaming serves to liberate bound ascorbic acid from the insoluble portions of the tomato. If the value for the "raw" sample had been based on the analysis of the whole tomato as obtained by blending sections of whole tomatoes with extracting solution, a more uniform basis would have been obtained for calculation of retentions; however, this procedure is objectionable in that portions of the tomato, such as seeds and skin, are included in the analysis, whereas they are excluded in the manufacture of tomato juice. Further work on this problem is needed.
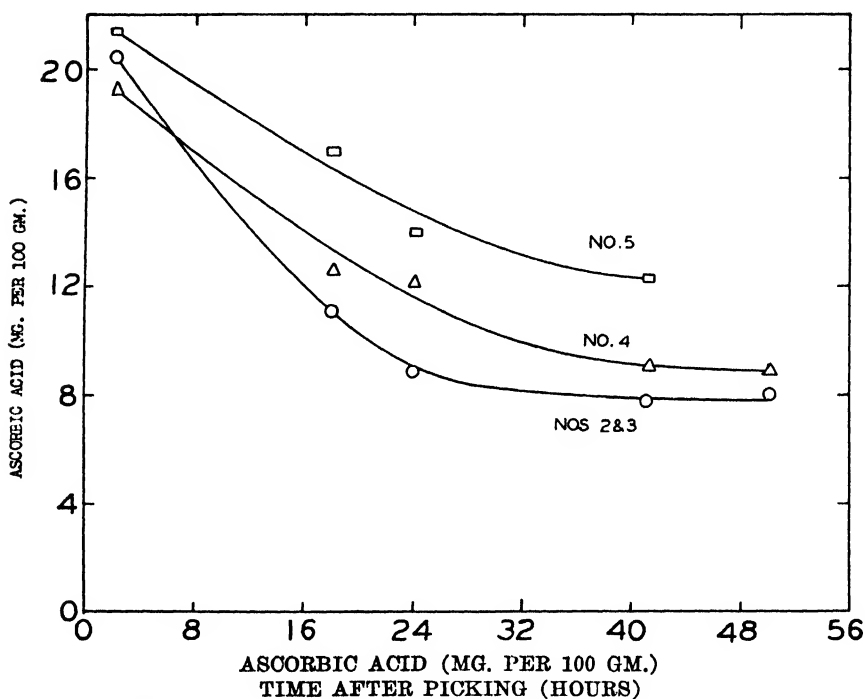
FIG. 1. Effect of holding on the ascorbic acid content of raw green beans, Blue Lake variety, of different sieve sizes.

The data indicate good retention of thiamin, riboflavin, and niacin in the canning of tomato juice. In most instances the slight variations found in these vitamins between different sampling points may be attributed to experimental error.

Loss of Nutrients Between Harvesting and Canning of Green Beans: A problem of considerable concern to food processors is the loss of nutrients between the time of harvesting and of canning. That such losses occur in green beans, peas, and spinach has been shown by several investigators— Mack, Tapley, and King (1939); Mack and Tressler (1936); and Tressler,

Mack, and King (1936). In conjunction with work on vitamin retention in canned green beans, experiments on the effect of holding raw beans of the Blue Lake variety prior to canning were performed. Of the vitamins tested, ascorbic acid was the only one showing an appreciable rate of loss during the period in which beans might be held prior to canning. Results obtained in a typical experiment are shown (Fig. 1); the temperature of holding varied from 18.9 to 21.1°C.(66 to 70°F.) in this experiment. A rapid loss of ascorbic acid occurs during the first 12 to 24 hours of holding and the proportion lost is greater in the small sieve sizes of beans than in the large sizes. No tests were made for dehydroascorbic acid, hence it is not known what proportion of the ascorbic acid lost was in the reversibly oxidized form; however, since dehydroascorbic acid is readily destroyed in canning, the presence of this substance in the raw product is unimportant from a canning standpoint, American Can Company (1943).

TABLE 4

Effect of Processing on Retention of Thiamin in Green Asparagus and Peas

Code	Product	Can size	Process		Thiamin—actual values	Retention during processing (dry basis)
			Time	Temperature		
			min.	*°F.*	*mg./100 gm.*	*pct.*
A-1	Asparagus	No. 2	15	248	.082	76
2	Asparagus	No. 2	25	248	.071	66
3	Asparagus	No. 2	25	240	.080	75
4	Asparagus	No. 2	40	240	.075	71
B-1	Asparagus	No. 2	15	248	.075	73
2	Asparagus	No. 2	25	240	.074	71
C-1	Peas	No. 2	18	252	.086	66
2	Peas	No. 2	23	252	.078	60
3	Peas	No. 2	30	252	.071	54
4	Peas	No. 10	18	252	.086	66
5	Peas	No. 10	23	252	.084	64
6	Peas	No. 10	30	252	.078	60

Effect of Processing on Thiamin Retention: It is observed that of the five vitamins included in these surveys only thiamin undergoes appreciable destruction as a result of processing. It became of interest to know whether the retention of thiamin could be altered to any appreciable extent by variation of processing times and temperatures. Data obtained in two separate experiments on asparagus and one experiment on peas are shown (Table 4).

In processing asparagus in No. 2 cans, 15 minutes at 120°C.(248°F.) is considered the equivalent in sterilizing value to 25 minutes at 115.6°C. (240°F.). It is seen that the destruction of thiamin is very nearly equal for the two cooks. In processing peas at the cannery in which these tests were made a cook of 18 minutes at 122.2°C.(252°F.) was given No. 2 cans and a cook of 30 minutes at 252°F. was given No. 10 cans. It is seen that slightly more thiamin was destroyed in the latter cook than in the

former. For the same cooks, however, more destruction occurred in the No. 2 cans than in the No. 10 cans, the difference becoming greater as the time was increased.

DISCUSSION

Of the vegetables included in this survey asparagus showed consistently the best retention of all the vitamins. This was probably due to the mild blanching given this product, its comparatively small surface area, and the unusual stability of its ascorbic acid. In California the great majority of canners use steam-blanching exclusively for asparagus. Its low ratio of surface area to weight results in a minimum of leaching out of water-soluble vitamins. Tests have shown, moreover, that the ascorbic acid in asparagus is not as readily oxidized as the ascorbic acid in certain other vegetables. Slightly better retention for asparagus was found than was reported by Wagner, Strong, and Elvehjem (1947) for midwestern asparagus. The fact that water-blanching was used in the canning operations studied by those investigators may account for the difference in retention.

In peas, green beans, and spinach blanching accounted for the principal loss of all vitamins except thiamin, which was lost primarily during processing. A considerable amount of ascorbic acid was lost between blanching and processing in green beans. This was due to the extremely unstable nature of the ascorbic acid in blanched beans. Peas appear to be somewhat more stable than green beans after blanching. This may be due to the more complete inactivation of enzymes in peas than in green beans during blanching. Although riboflavin and niacin are well retained in green beans, there is a considerable loss of niacin and a moderate loss of riboflavin in peas, most of which occurs during blanching.

The data show an apparent increase in the carotene content of both peas and carrots during the canning operations. It is suspected that the apparent increase in the case of peas is due to the leaching of soluble solids, which, as discussed by Bailey and Dutton (1945), indicates an increase in the concentration of carotene when results are expressed on the dry basis. This explanation does not account for the apparent increase in the carotene content of carrots after steaming, as determination of soluble and insoluble solids on these samples did not show that any appreciable amount of leaching had taken place. A possible explanation is that partial enzymatic destruction of carotene occurred during the preparation for analysis of the unheated samples. After steaming, the enzyme or enzymes responsible for this destruction may have been inactivated so as to cause no further destruction of carotene.

The data also show an apparent increase of ascorbic acid after processing in corn and to a lesser extent in spinach and clingstone peaches. The increase of ascorbic acid after processing has also been noted by Wagner, Strong, and Elvehjem (1947), who have suggested the possibility that ascorbic acid is liberated from a bound form during processing. They state, however, that the above explanation is open to question, presumably because it has not been clearly established that some interfering substance or substances are not formed during the processing of these products. Until this problem is solved, interpretation of the data on ascorbic acid retention in these products as a result of processing is difficult.

Loss of ascorbic acid in clingstone peaches is seen to occur both during lye-peeling and during the stages between lye-peeling and processing. Experiments performed subsequent to this work have demonstrated the instability of the ascorbic acid in freshly lye-peeled peaches. Steam-peeling of Elberta peaches appears to cause a considerable loss of ascorbic acid. A portion of this loss may be mechanical in nature since Schroder, Satterfield, and Holmes (1943) have shown that ascorbic acid is most concentrated in the skin of the peach and in the flesh adjacent to the skin.

Extensive work on the retention of ascorbic acid in tomato juice has demonstrated that this vitamin is rapidly destroyed when the juice is heated in the presence of air. Our data show that this loss is accelerated tremendously by the presence, in addition to air, of certain metallic salts, particularly copper. In the absence of air very little destruction of ascorbic acid can occur, as is evidenced by the almost complete retention of this vitamin in completely deaerated juice and during processing, providing there is a minimum of air incorporated in the can.

RECOMMENDATIONS FOR OBTAINING HIGH VITAMIN RETENTION

Examination of the data obtained as a result of this investigation leads to the following recommendations in order to obtain the maximum retention of nutrients in canned food:

1. In order to improve vitamin retention in canned vegetables, overblanching should be avoided and all blanching of vegetables should be kept to a minimum consistent with the production of a high-quality product.
2. Rapid handling of the raw product is essential to a maximum retention of the vitamin C present in the product at the time of harvesting.
3. Once canning operations are commenced they should be completed without delay and with minimum exposure to air. Particular attention should be given to the removal of air from juice products.
4. Metals, such as copper, which accelerate the oxidation of ascorbic acid should be kept at a minimum by avoiding wherever possible prolonged contact of the product with equipment containing these metals.

SUMMARY

Experiments have been performed on 12 canned products; namely, apricots, green asparagus, white asparagus, green beans, carrots, corn, clingstone peaches, freestone peaches, peas, spinach, tomatoes and tomato juice, in order to determine the retention of ascorbic acid, thiamin, riboflavin, niacin, and carotene during canning operations.

Excellent retention of all the vitamins except thiamin was obtained in asparagus. Water-soluble vitamins, especially ascorbic acid, were lost during the blanching of peas, green beans, and spinach. Additional losses of ascorbic acid between blanching and processing were observed in green beans and spinach, but in the other products these losses were negligible. Retentions of riboflavin and niacin were excellent except in the case of peas, in which appreciable amounts of both vitamins were lost in the blanch. Apparent increases of carotene were observed in carrots and peas.

Ascorbic acid appeared to increase during the processing of corn, peaches, and spinach.

No appreciable differences were observed between the retentions obtained on the same product at different canneries in any products except spinach and tomato juice, in which marked differences were observed in the retention of ascorbic acid. Variations in blanching procedures account for the differences in over-all retention of ascorbic acid in spinach. Aeration, time of holding, and possibly contamination with metals may account for differences in the retention of ascorbic acid in tomato juice. Good retention of thiamin, riboflavin, and niacin in tomato juice was obtained.

Processing had very little effect on any of the vitamins studied except thiamin. Ascorbic acid was not appreciably affected by processing, provided the incorporation of air in the can was not excessive. Thiamin was destroyed by processing, the magnitude of its destruction in a given product depending primarily on the time and temperature of processing.

Data are presented showing that prompt handling of the raw product is essential to obtaining good retention of ascorbic acid in green beans.

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ADDITION OF GROUND EGG SHELL TO DRIED EGG FOR USE IN COOKING

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In the production of dried egg the shells, which are calcium carbonate, are a waste product. If a way can be found to utilize the shells in a food product, the calcium content of the diet can be increased. Moreover, the calcium content of eggs is low in relation to their high content of phosphorus. Therefore, the addition of small amounts of ground egg shell to dried egg would play a significant role in correcting this low ratio of calcium to phosphorus. Since the addition of ground egg shell to dried whole egg may alter the quality characteristics of the food products in which dried egg is used, it seemed necessary to investigate this phase of the problem before commercial application of this method of utilizing a waste product could be recommended.[1] This paper reports the results of experiments on the flavor and cooking quality of dried egg with and without the addition of a small amount of ground egg shell.

Although the effect of adding ground egg shell to dried egg was hitherto unknown, some studies had been made using other forms of calcium carbonate, the chief ingredient of egg shell. It has been reported that calcium carbonate, when added to flour, caused accelerated bacterial growth resulting in a condition known as "rope" in bread by increasing the pH. However, Halton (1942) found that one per cent creta praeparata, a form of calcium carbonate, increased pH too little to have any practical significance. Commercial preparations containing calcium in the form of lime water are sold for the purpose of increasing foaming power of cream and egg whites.

Food products in which the various properties of egg play an essential role were selected for this study. Scrambled egg, baked custard, ice cream, foundation cake, muffins, yeast rolls, popovers, and mayonnaise were prepared. The effect of ground egg shell on the flavor and texture was evaluated and general preference indicated by a judging panel; the effect on the coagulating ability, stretching, binding, and emulsifying power of dried egg was measured by physical and chemical methods.

EXPERIMENTAL PROCEDURE

One hundred pounds of dried whole egg packaged in three-pound tins in an atmosphere of nitrogen were procured from a co-operating processing plant. The dried egg was stored at 0°C.(32°F.) for the duration of the

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[1] Investigations in feeding dried egg with shell have been carried out by the Bureau of Animal Industry.

experiment, as previous investigations by Dawson, Shank, Lynn, and Wood (1945) had indicated little deterioration at this temperature.

Ground egg shell was obtained from a commercial concern. Since the egg shell as received from the manufacturer was unevenly ground, it was sifted through sieves of different sizes, having U. S. sieve numbers 100, 140, 200, 325, and 400, with sieve openings of 0.0058, 0.0041, 0.0029, 0.0017, and 0.0015 inch, respectively.

The proportions of ingredients used in the preparation of scrambled egg, baked custard, ice cream, foundation cake, muffins, yeast rolls, popovers, and mayonnaise are listed (Table 1). The ingredients were combined by standard procedures. Two batches of each product were prepared, one containing dried egg with added ground egg shell and the other untreated dried egg for control. Before the egg was reconstituted, egg shell was added in 0.4 per cent concentration to the amount of dried egg needed for each product. To approximate one fresh egg, 13.5 grams of dried egg were reconstituted with 34.5 grams of water. The reconstituted egg was then combined with other ingredients in the same manner as fresh egg.

A well-insulated electric oven with a revolving hearth and accurate temperature control was used for all baking. The oven was calibrated in terms of temperature and baking performance.

Scrambled egg was prepared by two methods of cooking: (1) in a hot water bath, unseasoned, and (2) in a frying pan, seasoned, with and without bacon fat. The reconstituted egg coagulated by the first method contained unsifted ground egg shell and that sifted through each of the various sieves. Ground egg shell which had been sifted through No. 200 and No. 400 sieves was added to the egg prepared by the second method. Paired samples, one with and one without ground egg shell, were presented to each member of a judging panel for evaluation of quality.

Custards were baked in 50-milliliter beakers, 24 at a time, 12 with and 12 without egg shell. The custards were cooled before presentation to a judging panel. Height measurements were taken with the aid of the penetrometer after baked custards had stood two hours at room temperature.

Ice cream was frozen in a household refrigerator. Each batch was poured into a separate tray and stirred at 30-minute intervals until firm. Paired samples in an ice bath were presented to each judge.

Foundation cake was made by the conventional method; an electric mixer was used for combining ingredients. Four cakes with egg shell were baked at the same time, immediately after which four cakes without egg shell were baked. Volume was measured by the seed displacement method on the cooled cakes in the pans in which they were baked. Crusts were removed from the cakes and six three-inch by one-inch pieces were cut from each cake. Pieces from the control were matched with pieces from corresponding positions in the samples containing egg shell. These pairs were judged by the panel.

Muffins were mixed by a standard procedure whereby liquid ingredients were stirred into the dry ingredients. Forty-eight muffins in four pans were baked at one time, 24 with and 24 without the addition of ground egg shell. Paired halves were judged while hot.

TABLE 1

Ingredients Used in Preparation of Food Products

Ingredients	Scrambled egg		Baked custard	Ice cream	Founda-tion cake	Muffins	Yeast rolls	Popovers	Mayon-naise
	Unseasoned	Seasoned							
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Ground egg shell	0.054	0.324	0.162	0.054	0.108	0.054	0.108	0.108	0.054
Dried whole egg	13.5	81.0	40.5	13.5	27.0	13.5	27.0	27.0	13.5
Water (distilled)	34.5	220.5	103.5	34.5 (28.0)	69.0	34.5	69.0 (60.0)	69.0	34.5
Milk	......	81.0	488.0	244.0	162.5	244.0	183.0	244.0	......
Sugar	......	......	38.0	100.0	200.0	25.0	25.0	......	4.0
Fat (hydrogenated)	......	......	......	......	100.0	25.0	25.0	24.0	......
Flour (all-purpose)	......	......	......	7.0	......	220.0	385.0	110.0	......
Flour (cake)	......	......	......	......	192.0	......	......	......	......
Baking powder (S.A.S.)	......	......	......	......	12.0	12.0	......	......	......
Sodium chloride	......	3.0	1.0	1.5	1.5	3.0	12.0	2.0	3.0
Pepper	......	Dash	.	......	......	......	......	......	......
Vanilla	......	......	......	1 tsp.	......	......	......	......	......
Oil (corn)	......	......	......	......	......	......	......	......	220.0
Bacon fat	......	41.0	......	......	......	......	......	......	......
Vinegar	......	......	......	......	......	......	......	......	20.0
Gelatin	......	......	......	3.5	......	......	......	......	......
Yeast, compressed	......	......	......	......	......	......	24.0	......	......
Cream (40%)	......	......	......	354.0	......	......	......	......	......

Yeast rolls were prepared at room temperature and the dough was allowed to rise in a proof box at 31°C.(88°F.) once before and once after the rolls were shaped. Two laboratory technicians working at the same time shaped rolls from 25 grams of dough. Four pans, two with treated and two with untreated rolls, containing 14 rolls in each pan, were baked at the same time. Paired hot samples were tasted by a panel of judges. Determinations of hydrogen-ion concentration were made with a Coleman pH electrometer directly on the inner portion of a baked cooled roll which had been worked between the fingers until doughy.

Popovers made with and without ground egg shell were baked at the same time in two iron pans, each containing 10 samples, for 15 minutes at 232°C.(450°F.) and then for 30 minutes more with the thermostat lowered to 176.7°C.(350°F.). Volume comparisons were made by the seed displacement method after popovers were removed from pans.

Mayonnaise was made by mixing dry ingredients with one-half of the vinegar and combining this with the reconstituted whole egg before the oil was added. The remaining oil and vinegar were beaten in alternately with the aid of an electric mixer. Mayonnaise was allowed to stand for four hours at room temperature before being tested for consistency differences. The penetrometer devised by Hall and Dawson (1940) was used for measuring the distance of penetration of a plummet into each mayonnaise sample.

The taste panel consisted of six to 10 judges who evaluated paired samples of scrambled egg, baked custard, ice cream, cake, muffins, and yeast rolls for flavor and texture and indicated which one they preferred in general. In some products the presence or absence of grittiness was specifically noted. Judgments were recorded on a score card (see below) which offered a triple choice for decision: Sample A is superior to Sample B; Sample A is inferior to Sample B; or there is no difference between Samples A and B. The results were evaluated statistically by the chi-square method, testing the 50:50 hypothesis. A similar method of "paired-eating" was used by Sylvia Cover (1940) in meat-cookery research.

SCORE CARD

Name_____ Have you tasted before today?_____
                                                        yes   no
Date_____ If so, when and what?_____

Time_____   _____

State of health: Excellent   Remarks:
                 Fair
                 Poor

Choice for decision	Sample A	Sample B	No difference
Which of the 2 samples— Has better flavor?			
Has better texture?			
Contains grittiness?			
Do you prefer?			

The significance of the difference between means of the physical and chemical measurements was determined by the t-test.

RESULTS AND DISCUSSION

The particle size of the ground egg shell available for this study varied considerably. Only 66 per cent went through a U. S. No. 400 sieve having a sieve opening of 0.0015 inch, whereas 97 per cent went through a No. 100 sieve having a sieve opening of 0.0058 inch (Table 2).

TABLE 2
Yield of Ground Egg Shell of Different Particle Size

Sieve size— U. S. No.	Sieve opening	Ground egg shell that went through sieve	Residue after sifting
	in.	*pct.*	*pct.*
100	.0058	97	3
140	.0041	93	7
200	.0029	87	13
325	.0017	82	18
400	.0015	66	34

The residue remaining consisted of coarse particles of egg shell and cotton-like material from the shell membrane. The unsieved material had an undesirable odor which was not noticeable in the shell sifted through even the coarsest sieve. Hence, the odor was attributed to the membranous material. Removal of this part of the egg shell by sifting would help to prevent a contamination of the flavor of food products in which egg shell is used.

The judgments of the taste panel with respect to the effect of ground egg shell on the palatability of the several food products are summarized (Tables 3 and 4); the effect of egg shell on cooking quality of the products as determined by physical and chemical tests is shown (Table 5).

SCRAMBLED EGG

In preliminary laboratory tests an undesirable grittiness was detected when the unsifted product was used in scrambled egg cooked unseasoned in the hot-water bath. Three tasters in the laboratory also could detect grittiness in the samples with added egg shell sifted through sieves varying in size from U. S. No. 100 to 325. When the egg shell was sifted through the No. 400 sieve, which has an opening of 0.0015 inch, it was fine enough to escape detection by two out of three tasters in the laboratory.

When submitted to the judging panel, no significant differences were noted in scrambled egg made with and without ground egg shell of No. 400 sieve size. Although a few of the judges preferred the sample with ground egg shell, the majority indicated no difference in flavor, grittiness, or general preference.

Grittiness was not detected with egg shell of No. 200 sieve size when bacon fat was added although egg shell of this size was easily detected when bacon fat was not used, as mentioned above in the preliminary tests and as later confirmed by the judging panel. Apparently the flavor and lubricating effect of the fat interfered with the judges' ability to detect grittiness.

TABLE 3

Effect of Ground Egg Shell on Palatability of Several Food Products Made With Dried Whole Egg as Determined by a Taste Panel

Food product	Sieve-size of shell used—No.	Total tests on paired samples[1]	Choice for decision	Flavor		Texture		Presence of grittiness		General preference	
				Votes	Chi-square[2]	Votes	Chi-square[2]	Votes	Chi-square[2]	Votes	Chi-square[2]
Scrambled egg (without bacon fat)	400	21	With egg shell	4		...		4		4	
			Without egg shell	4	0.00	...	......	1	0.43	5	0.05
			No difference	13		...		16		12	
Baked custard	140	63	With egg shell	13		...		12		19	
			Without egg shell	19	0.57	...	......	8	0.25	22	0.14
			No difference	31		...		43		22	
Ice cream	140	84	With egg shell	17		17		....		16	
			Without egg shell	19	0.57	19	......	....	.....	19	0.11
			No difference	48		48		....		49	
Foundation cake	100	63	With egg shell	22		29		....		28	
			Without egg shell	19	0.05	25	0.05	....	.....	28	0.00
			No difference	22		9		....		7	
Muffins	100	69	With egg shell	27		23		....		29	
			Without egg shell	17	0.14	33	0.25	....	.....	23	0.00
			No difference	25		13		....		17	
Yeast rolls	140	63	With egg shell	8		14		18		12	
			Without egg shell	15	1.45	20	1.45	17	......	17	0.52
			No difference	40		29		28		34	
					0.78		0.57		0.02		0.40

[1] Tested by a panel of five to nine judges on replicate samples. [2] A chi-square of 3.84 is necessary for significance at the five-per cent level.

TABLE 4

Effect of Bacon Fat and Particle Size of Ground Egg Shell on Palatability of Scrambled Egg as Determined by a Taste Panel

Sieve-size of shell used—No.	Method of cooking	Total tests on paired samples[1]	Choice for decision	Flavor		Presence of grittiness		General preference	
				Votes	Chi-square	Votes	Chi-square	Votes	Chi-square
200	With bacon fat	18	With egg shell	0		10		3	
			Without egg shell	3	0.50	3	2.72	7	0.89
			No difference	15		5		8	
200	Without bacon fat	21	With egg shell	5		17		2	
			Without egg shell	9	0.76	0	13.76[2]	16	9.33[2]
			No difference	7		4		3	
400	With bacon fat	21	With egg shell	2		5		2	
			Without egg shell	3	0.05	3	0.19	3	0.05
			No difference	16		13		16	
400	Without bacon fat	21	With egg shell	4		4		4	
			Without egg shell	4	0.00	1	0.43	5	0.05
			No difference	13		16		12	

[1] Tested by a panel of six to seven judges on replicate samples.   [2] Significant.

Grittiness was not noticed when the more finely ground shell of No. 400 sieve size was used either with or without bacon fat.

BAKED CUSTARD

In baked custard, ground egg shell of 140 sieve size was not recognized as gritty by the panel and, therefore, the test was not repeated with egg shell of finer granulation. Even though baked custard has a smooth texture in which any rough particles of egg shell would be noticeable, under the conditions of this experiment the large quantity of liquid in the recipe apparently was capable of dissolving the calcium carbonate of egg shell during the long baking period. The flavor of the product and general preference as indicated by the panel were not affected by the addition of ground egg shell to dried egg used in baked custard.

TABLE 5

Effect of Ground Egg Shell[1] on Cooking Quality of Dried Whole Egg as Determined by Physical and Chemical Tests

Quality studied	Variable	Number of tests	Average	Standard deviation	"t" value
Height of baked custard	With egg shell	36	30.0 mm.	0.65	
	Without egg shell	36	30.3 mm.	1.16	
					1.26
Volume of foundation cake	With egg shell	12	634 ml.	9.3	
	Without egg shell	12	644 ml.	14.9	
					1.97
pH of yeast rolls	With egg shell	6	5.80 pH	0.037	
	Without egg shell	6	5.74 pH	0.048	
					2.43[2]
Volume of popovers	With egg shell	60	201 ml.	11.1	
	Without egg shell	60	194 ml.	11.3	
					3.00[3]
Consistency of mayonnaise	With egg shell	9	3.49 cm.	0.13	
	Without egg shell	9	3.29 cm.	0.11	
					3.51[3]

[1] Unsieved egg shell was used in popovers, cake, custard, and mayonnaise; No. 150 sieve size was used for yeast rolls. [2] Significant at the five-per cent level. [3] Significant at the one-per cent level.

The coagulating ability of dried egg, as measured by the height of baked custards, was not altered favorably or unfavorably by addition of ground egg shell. The average heights for 36 samples with ground shell and 36 samples without ground shell were 30 and 30.3 millimeters, respectively, (Table 5).

ICE CREAM

The results of 84 tests by a panel of nine to 10 judges indicated that ground egg shell of No. 140 sieve size has no effect on the flavor or texture of vanilla refrigerator ice cream. Most of the judges noted no difference in these quality characteristics when paired samples, with and without ground egg shell, were tested.

FOUNDATION CAKE AND MUFFINS

In the preliminary tests there was an indication that the addition of unsifted ground egg shell had a detrimental effect on the texture of founda-

tion cake made with dried egg. However, further tests by a panel of seven judges on over 300 paired samples of cake made with and without ground egg shell of No. 100 sieve size revealed no significant differences in texture or in flavor and general preference. The average volume of 12 cakes made with added ground egg shell was 634 milliliters as compared with an average volume of 644 milliliters for 12 cakes without shell, a difference which was statistically insignificant.

The use of ground egg shell of No. 100 sieve size in muffins likewise produced insignificant differences in flavor, texture, and general preference as noted by the judging panel.

YEAST ROLLS

Yeast rolls made without and with ground egg shell of No. 140 sieve size appeared to be very similar in external color of the crust and in size. When paired samples were judged by the panel, about 50 per cent of the opinions indicated no differences in the flavor or texture of the products. The remainder of the votes for samples with or without egg shell canceled each other so that any differences which the judging panel noted were found to be statistically insignificant.

The calcium carbonate of egg shell at the 0.4 per cent concentration raised the pH of the crumb from 5.74 to 5.80. This slight increase in pH was not enough to affect the stability of any vitamins present or cause any appreciable acceleration of "rope" development.

POPOVERS

The average volume of popovers made with ground egg shell was significantly larger than the volume of controls made without ground egg shell. This is an advantage since the desirability of popovers is directly proportional to the volume, and the use of dried egg results in slightly smaller popover volumes than obtained with fresh shell eggs, Dawson, Wood, and McNally (1946). The addition of ground egg shell makes possible the production of popovers with volumes more nearly like those made with fresh eggs.

MAYONNAISE

The addition of 0.4 per cent ground egg shell decreased slightly the emulsifying power of dried whole egg. The average depth of penetration of the plummet in the mayonnaise emulsion made with ground egg shell was slightly greater than in the control, or 3.49 and 3.29 cm., respectively, a difference which was statistically significant. The calcium in ground egg shell is antagonistic to sodium present in salt when both are in solution, and may prohibit emulsification if in high enough concentration. However, the product was only slightly thinner and was acceptable for use.

SUMMARY

. Scrambled egg, baked custard, ice cream, foundation cake, muffins, yeast rolls, popovers, and mayonnaise were prepared for a study of the utilization of dried egg fortified with calcium from ground egg shell. Samples of each product made with and without the addition to the dried egg of 0.4 per cent

ground egg shell were compared by sensory, physical, and chemical criteria.

It was found that the addition of ground egg shell in the concentration used did not affect the palatability or cooking quality of dried egg in most food products, provided the shell was ground finely enough to pass through a U. S. No. 400 sieve with openings of 0.0015 inch. Particles of egg shell coarser than this resulted in grittiness in scrambled eggs. Grittiness was not noticed in any of the other products, probably owing to the presence of other ingredients and longer cooking times. Ground egg shell decreased the thickness of mayonnaise and increased the volume of popovers.

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A NOTE ON DETERMINATION OF THIAMIN BY FUNGUS-GROWTH METHOD

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The use of *Phycomyces blakesleeanus* for the quantitative assay of thiamin, described by Hamner, Stewart, and Matrone (1943), has proved to be efficient and reliable in this laboratory in analyses of fruits and vegetables. The cost of equipment to carry out the procedure is far less than that required in the chemical, thiochrome procedure. Many laboratories do not have the requisite photofluorometer. In the fungus-growth method all the chemicals used are inexpensive except for asparagine, which is not the case in the thiochrome method.

Briefly, the fungus is grown on 25 ml. of a medium composed of mineral salts, water, dextrose, and asparagine in 125-ml. conical flasks. After two weeks, the mycelial mats produced by growth of the fungus are dried and weighed, and the thiamin contents of vegetable extract are read from a standard curve simultaneously produced by growth of the fungus in flasks to which known amounts of a pure thiamin solution have been added. All samples and standards (0.1 to 0.6 microgram of thiamin per flask) are carried out with four to six replicates. Since the thiamin content of a vegetable can vary widely, its extract is usually added at two levels, for instance, two and four ml.; five grams of asparagine are used per liter of medium in this procedure.

Where large numbers of samples are analyzed, the cost of the asparagine is considerable. Glycine, a simpler and far less expensive amino acid, was substituted, therefore, for the asparagine on an experimental basis.

Seven and one-half grams of glycine were substituted for the five grams of asparagine, and assays were carried out otherwise exactly according to Hamner *et al.* (1943). The experiment was repeated three times. The fungus grew well on the glycine medium to which vegetable extracts had been added. It was found, however, that the response by the fungus to thiamin in the vegetable extracts was not entirely satisfactory because (1) pure thiamin added to the extracts gave more than 100 per cent recoveries and (2) the concentration of thiamin read for a four-ml. aliquot was more than double that found for a two-ml. aliquot of the vegetable extract. In several cases, furthermore, the fungus grew normally when supplied with vegetable extract, but little if any when only pure thiamin solutions were added to the medium.

It was next observed that one could use glycine in the medium to which were added the extracts of vegetables to be analyzed, while using asparagine only for the medium used in preparing the standard curve. With this

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procedure, results were obtained substantially identical with those obtained when asparagine was used in the vegetable extract medium. Analytical results obtained by comparison with a standard curve derived from an asparagine medium by (a) vegetable extracts grown on an asparagine medium and (b) extracts grown on a glycine medium are given (Table 1).

TABLE 1

Thiamin Content of Foods Determined by Fungus-Growth Method

Food	Asparagine medium	Glycine medium [1]
	µg./100 gm.	*µg./100 gm.*
Frozen lima beans..	97	100
Frozen lima beans..	97	95
Frozen lima beans..	87	87
Frozen lima beans..	131	137
Frozen peaches...	13.8	12.2
Frozen peaches...	16.5	20
Frozen peaches...	13.1	15.2
Cooked tomatoes...	91	91
Cooked tomatoes...	78	66

[1] Vegetable extracts were added to a medium containing glycine but were compared with standard curves run with an asparagine medium. See text for explanation.

It was further noted that, where both two- and four-ml. aliquots of the vegetable extract fell on the curve, the thiamin contents for the vegetable were identical; that is, the response to four ml. was exactly twice that obtained when two ml. were used. It will be seen that the results obtained on the two types of media were in good agreement. This phenomenon is probably due to the presence in the fruits and vegetables of an excess of organic nitrogen of a type utilizable by the fungus. When supplied with this nitrogen, the glycine medium is adequate; the factor limiting growth, therefore, appears to be thiamin. On the other hand, the standard fulfills the same requirement only when asparagine and not glycine alone is present.

The use of glycine in the medium added to the vegetable extracts, while retaining the asparagine in the extracts used for the standard curve, has the advantage of far lower cost and further superiority in that it can be obtained completely thiamin-free, while asparagine used in this laboratory often has been found to contain some thiamin which must be corrected by a blank determination.

SUMMARY

The fungus-growth method for determining thiamin in fruits and vegetables has been found to be satisfactory in the analysis of approximately 2,000 samples in the laboratory. It was found that glycine, a far cheaper amino acid, could be substituted for asparagine in the growth medium to which the vegetable extracts are added. It is necessary to use asparagine only in the standard calibration employing pure thiamin.

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A STUDY OF OXIDIZING ENZYMES OF GUAVA

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The guava (*Psidium guajava*, L.) is an unusually rich source of ascorbic acid. Golberg and Levy (1941) found that ripe and firm guava fruits contained 300 to 400 mg. of ascorbic acid per 100 grams of fresh fruit. Boyes and de Villiers (1942) reported higher values, over 600 mg. per 100 gm. of fresh fruit. Guava pulp, therefore, has been considered for fortifying fruit bars in Army rations. Because there are considerable losses on storage, a study of the oxidizing enzymes is of interest, and it is the purpose of this paper to consider the enzymatic factors that may be involved.

Three common enzyme systems in plants, according to Green (1940), catalyze the oxidation of ascorbic acid directly or indirectly: ascorbic acid oxidase, peroxidase, and phenol oxidases (phenolases). Also, some other plant enzymes, e.g., the cytochrome oxidase system and laccase, may be capable of oxidizing ascorbic acid.

EXPERIMENTAL PROCEDURE

The ascorbic acid content of guavas was previously determined by Ballentine's method (1941). The results obtained showed that it ranged from a low of 55 mg. per 100 gm. to a high of 442 mg., depending on the variety.

To determine the retention of ascorbic acid in guavas in storage at —18°C. (—0.4°F.) samples were prepared in the following manner: The guavas were washed, the stem and calyx ends were cut off and discarded, and the halved fruit passed through an American Utensil Company tomato juicer. This mixture of outer flesh and skin was stored in small sealed bottles at —18°C. A second batch was prepared, but in this instance the halved fruits were blanched in steam for 10 minutes before they were passed through the tomato juicer and then placed in small sealed bottles and stored at the same temperature.

Samples were analyzed after they were passed through the juicer and each month while they were stored at —18°C. The initial amount of ascorbic acid in the raw pulp decreased from 354 mg. per 100 gm. of pulp to 227 mg. per 100 gm. after 115 days of storage. This shows 36 per cent loss in reduced ascorbic acid. The blanched samples, which originally contained 228 mg. ascorbic acid per 100 gm. of pulp, had, after 105 days of storage, 211 mg. per 100 gm. This represents a loss of 7.5 per cent in reduced ascorbic acid, which may be within the error of sampling.

The total ascorbic acid (reduced plus dehydroascorbic acid) present in the samples was determined by H_2S reduction. The results showed that in the raw samples, 6.7 per cent of the total was in the form of dehydro-

ascorbic acid after 100 days in storage at —18°C., and in the blanched samples, 9.4 per cent after 90 days of storage under similar conditions. As reported by Cruess, Hohl, Jiménez, Nichols-Roy, Torres, and Zorrilla (1945), loss in ascorbic acid was also observed when the fruit was stored in syrup at —18°C. for several days.

OXIDIZING ENZYMES PRESENT

Qualitative Tests: Ground outer flesh and also an enzyme extract of such flesh were tested on numerous substrates. The procedure used in preparing the enzyme extract was similar to that used by Ponting (1944) with other fruits. Fifty grams of peeled outer flesh were blended for two or three minutes with 150 ml. of ice water and filtered through coarse filter paper. The enzymes present were precipitated by adding two volumes of acetone at —23°C.(—9.4°F.). After standing 10 minutes the enzyme precipitate was filtered, dissolved in 50 ml. of ice water, and precipitated with two volumes of acetone at —23°C. After an additional 10 minutes the precipitate was filtered again and resuspended in 25 ml. of ice water, yielding a white suspension. This enzyme extract was used within a few days of preparation.

Positive tests were obtained with catechol, pyrogallol, p-phenylenediamine, intensified when hydrogen peroxide was added. Negative results were obtained with the monohydro-phenolic compounds, and with resorcinol and phloroglucinol, even in the presence of hydrogen peroxide.

LOCATION OF ENZYME IN GUAVA

To observe distribution of the enzyme, slices of the firm ripe guava were tested with four substrates: guaiacol, catechol, benzidine, and p-phenylenediamine. The last one seemed to be the best indicator for this test since the color was developed in a relatively short time and it was not too intense.

Microscopic observations on the location of the enzyme were made on free-hand sections since the consistency of the fruit and the presence of numerous stone cells and seeds made microtone sections almost impossible.

The highest oxidase activity seemed to be localized in the inner flesh, which is in agreement with the findings of Crist and Batjer (1931) in pears. Also the vascular bundles, especially the tissues surrounding the fibrovascular bundles, showed high enzyme activity. Samisch (1935) reported a similar location for the oxidase present in apricots, and more recently Hussein and Cruess (1940) made the same observation in grapes. The peel showed almost no activity (Fig. 1).

DISTRIBUTION OF ASCORBIC ACID IN RELATION TO ENZYME ACTIVITY

The concentration of ascorbic acid in guavas is not uniform but decreases gradually from the flesh next to the skin inward. Golberg and Levy (1941), Boyes and de Villiers (1942), and Webber (1944) have reported a similar variation.

Since the greatest enzyme activity was found in the inner flesh, and weak or negative reactions were found in the outer flesh and peel, respec-

tively, there appears to be an inverse relationship between the ascorbic acid content and enzyme activity.

PEROXIDASE ACTIVITY

An Evelyn photoelectric colorimeter was used to measure the effect of different factors upon the activity of peroxidase. Guaiacol was chosen as substrate because of its stability. The acetate buffer has the advantage that the tetraguaiacol formed as a product of the oxidation of guaiacol in the presence of the enzyme extract is completely dissolved, thereby giving a very clear solution. A 420 mμ filter was used in this work.
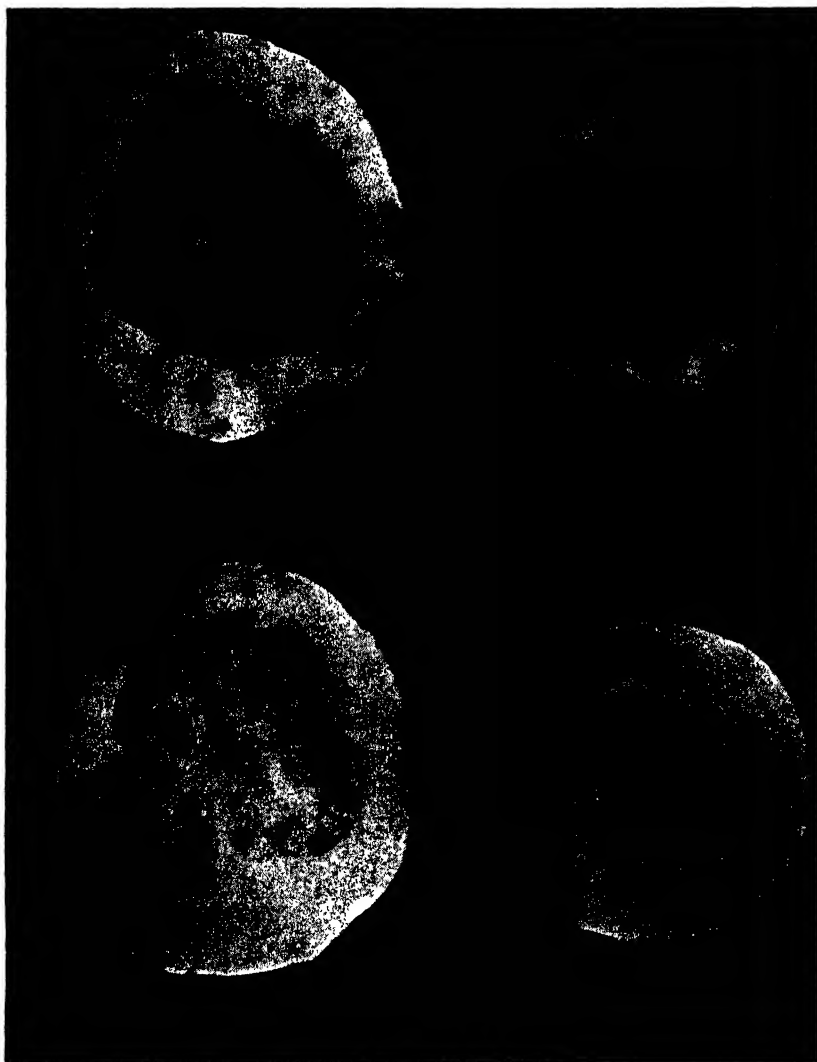
FIG. 1. Enzyme activity in guava fruits—1 and 3 show two different varieties treated with the indicator; 2 and 4 show the same varieties without any treatment.

pH : To determine the effect of pH on the activity of the guava enzyme extract several tests were conducted in acetate buffer solutions of different pH's. The range of the highest activity was from pH 5.0 to 6.0, and the optimum was pH 5.5 (Fig. 2).
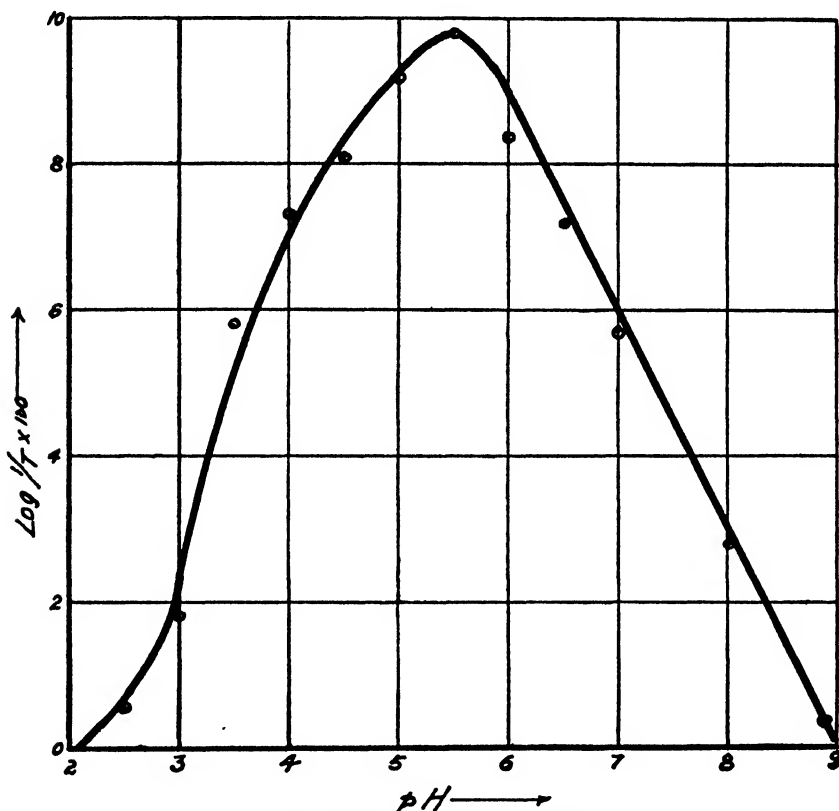
FIG. 2. Effect of pH on activity of guava enzyme.

Substrate Concentration: The effect of concentration of substrate upon the rate at pH 5.5 is shown by plotting log $\frac{1}{T}$ against per cent guaiacol (Fig. 3). Up to 0.75 per cent guaiacol concentration, the rate of the reaction varied almost directly with the substrate concentration, but above this, the activity decreased rapidly, which is in agreement with the Michalis-Menten law.

H_2O_2 Concentration: Under the conditions of this experiment the optimum hydrogen peroxide concentration was 2.5×10^{-3} mol. per liter, which is quite close to that found by Cruess and Fong (1929) for apricot oxidase. In the presence of an excess of hydrogen peroxide the enzyme was either destroyed or irrevocably bound (Fig. 4).

Cyanide and Fluoride: Peroxidase is not especially sensitive to heavy metal ions, but is inactivated readily by potassium cyanide. As shown in

Fig. 5, 84 per cent of the activity was destroyed by 5.5×10^{-6} mol. of KCN per liter.

The action of fluoride ion on the guava enzyme preparation showed that complete inhibition in the presence of sodium fluoride required a concentration of 7×10^{-2} mol. of NaF per liter.
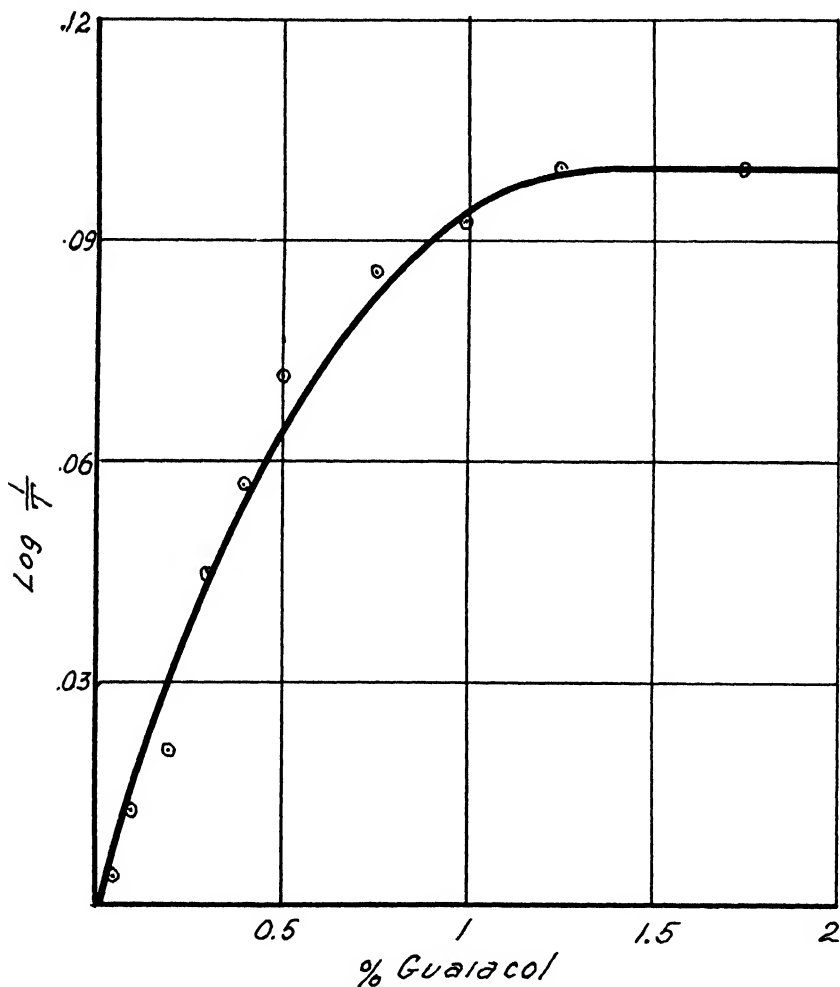
FIG. 3. Effect of substrate concentration on activity.

Temperature: There was a gradual increase of activity between 5 and 50°C. (41 and 122°F.), after which there was a thermal destruction of the enzyme (Fig. 6).

DEHYDROGENASE ACTIVITY

Experiments were conducted to determine whether or not dehydrogenases are present in guava tissues.

Two different substances were used as substrates: ascorbic acid and citric acid. Thunberg tubes were used to ascertain if the tissue or the

juice would oxidize any of the substances in the absence of air, with methylene blue as hydrogen acceptor. There was no decoloration of methylene blue, even after 24 hours, in the presence of either of the two substances. This showed that neither ascorbic acid dehydrogenase nor citric acid dehydrogenase appears to be present in guava flesh.

ENZYME ACTIVITY—ASCORBIC ACID AS SUBSTRATE

Ascorbic acid has been employed as a substrate in the determination of oxidase activity in fruits and vegetables by Ezell and Gerhardt (1940)
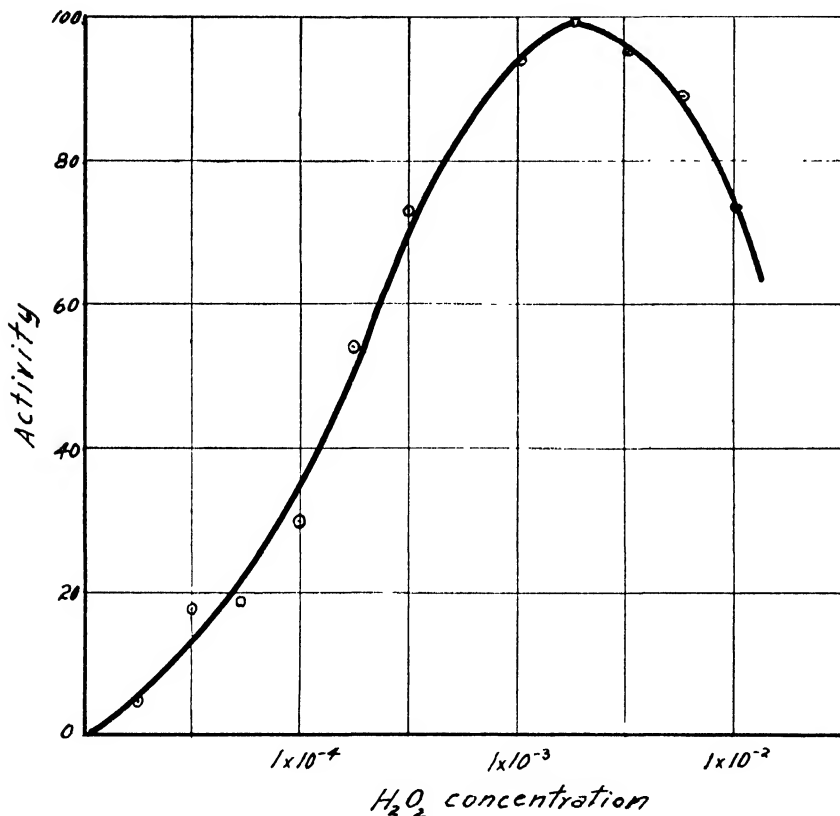
FIG. 4. Effect of H_2O_2 concentration on activity.

and Sreerangachar (1943). Experiments were conducted to determine the action of guava and enzyme extracts on ascorbic acid under different conditions. Water redistilled in Pyrex glass was used in all experiments. The Evelyn photoelectric colorimeter was again employed. Because the guava extract had a rather low activity with respect to ascorbic acid, Evelyn's conditions — Evelyn, Malloy, and Rosen (1938) — required modification, necessitating determination of a new constant. As check, however, Evelyn's constant was redetermined also.

Evelyn's constant k is derived from the formula k (L'—L″) = mg. ascorbic acid per 100 ml. of solution, from which Evelyn obtained a value of

10.8 for k (10.89 in our redetermination). Under the present conditions, a constant of 4.70 was used replacing Evelyn's 10.8.

The new constant was determined as follows: A solution of ascorbic acid, approximately 50 mg. per 100 ml., was prepared (Solution A). Ten ml. of Solution A was equivalent to 9.30 ml. of 0.0056 N iodine solution, whence Solution A contained 45.8 mg. per 100 ml. Solution A was then diluted as follows: Four ml. of Solution A plus 40 ml. of 0.1 M citrate-

KCN, molality
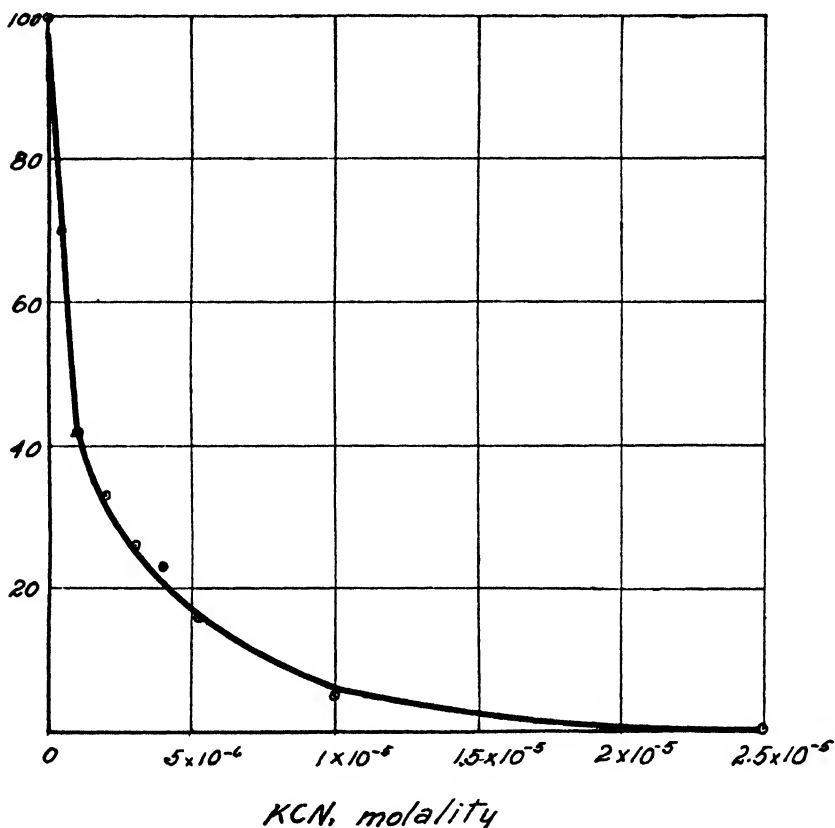
FIG. 5. Effect of KCN on activity.

oxalate buffer solution plus two ml. of M oxalic acid were made to 100 ml. (Solution B), which therefore contained 1.83 mg. of ascorbic acid per 100 ml. of solution.

Thirteen ml. of dye plus one ml. of oxalic acid plus three ml. of water gave a transmission reading of 0.285 (T), equivalent to an optical density of 0.545 (L').

(a) 13 ml. of dye plus two ml. of Solution B plus two ml. of water = 0.450 (T), equivalent to 0.347 (L'').

(b) 13 ml. of dye plus four ml. of Solution B = 0.690 (T), equivalent to 0.161 (L'').

(c) 13 ml. of dye plus four ml. of Solution C (2.29 mg. ascorbic acid per 100 ml.)

= 0.870 (T), equivalent to 0.061 (L″).

Whence

(a) $k = \dfrac{1.83}{0.198} = 9.24 = 2 \times 4.62$

(b) $k = \dfrac{1.83}{0.384} = 4.76$

(c) $k = \dfrac{2.29}{0.486} = 4.71$

The average value for the constant k is 4.70.
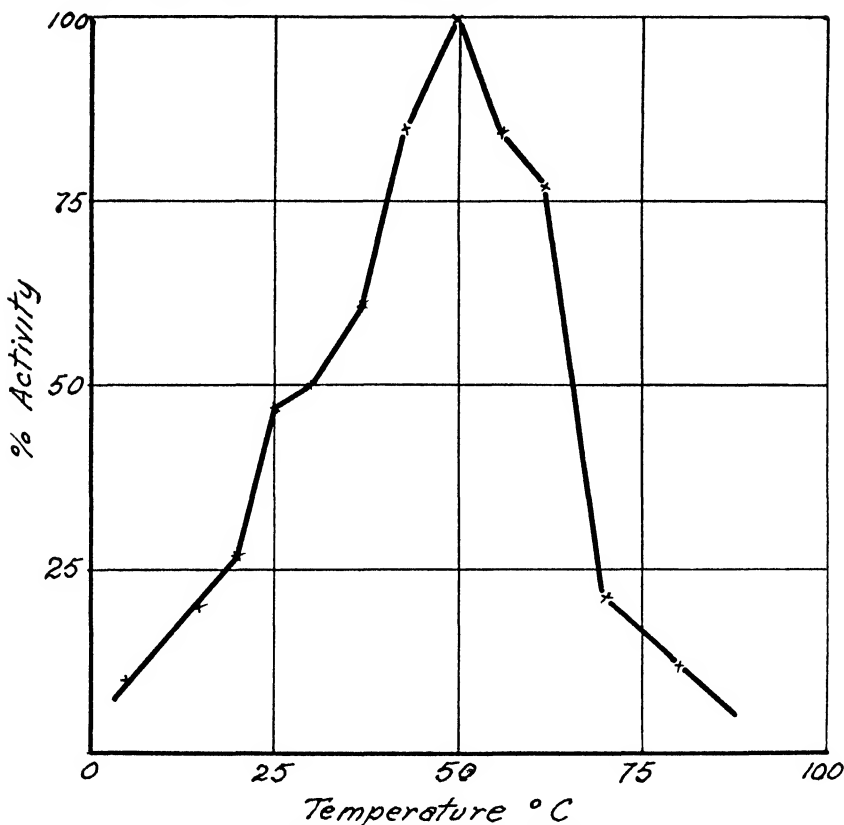
FIG. 6. Effect of temperature on enzyme activity.

Effect of Guava Extract on Ascorbic Acid: A guava extract was prepared by blending 20 grams of guava outer flesh with 200 ml. of 0.1 M oxalate-citrate buffer solution, pH 5.6. This was used either directly or after diluting 1:4.

Solutions were prepared as follows: Forty ml. of 0.1 M oxalate-citrate buffer solution, pH 5.6, containing the ascorbic acid previously added, were

pipetted into a 100-ml. volumetric flask, then two ml. of M oxalic acid were added and the mixture made to volume with distilled water. Four ml. of this solution was added to 13 ml. of the dye in duplicate, and a reading was taken after 15 seconds.

In the other tests, six ml. of the guava extract was added initially and the flask was placed in a water bath at 25°C.(77°F.) for attainment of temperature before the addition of the oxalic acid. The addition of the guava extract increased slightly the ascorbic acid content in some cases. After one hour a loss of about 20 per cent was observed.

TABLE 1

Effect of Guava and Guava Enzyme Extract on Ascorbic Acid

Test solution	Ascorbic acid oxidized per 100 ml. buffer solution at 25°C.				
	Diluted guava		Conc. guava	Enzyme	
	1	2	3	4	5
I. Initial plus ascorbic acid present in extract....................................	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
	1.43	1.33	2.02	1.16	1.42
II. (a) Loss in one hour.................................	0.05	0.33	0.27	0.04	0.24
(b) Loss using boiled extract..................	0.00	0.00	0.00	0.00	0.00
III. Plus catechol					
(a) Loss in one hour.................................	0.57	0.64	0.86	0.44	0.43
(b) Loss using boiled extract..................	0.00	0.00	0.00	0.00	0.00
IV. Plus catechol and H_2O_2					
(a) Loss in one hour.................................	0.76	1.33	2.02	1.08	1.42
(b) Loss using boiled enzyme.................	0.00	0.00	0.00	0.00	0.00
V. Plus hydrogen peroxide					
(a) Loss in one hour.................................	......	......	0.36	0.05	0.22
VI. Controls (without guava or enzyme extract); loss in one hour					
(a) Catechol..	0.00	0.00	0.00	0.02	0.00
(b) Catechol + H_2O_2................................	0.00	0.00	0.00	0.00	0.00
(c) Hydrogen peroxide............................	0.00	0.00	0.00	0.00	0.00

As shown (Table 1, Columns 1, 2, and 3) the addition of catechol caused an average loss of about 40 per cent. The amount of catechol used in each of these tests was eight mg., which, as previously determined, produced substantial oxidation of the ascorbic acid. The effect of catechol plus hydrogen peroxide was also considered. Hydrogen peroxide was added to give a concentration of 2.2×10^{-3} mol. per liter, about the optimum found in previous experiments, and this caused almost complete destruction. However, with guava extract to which only hydrogen peroxide had been added, about 18 per cent of the ascorbic acid present was lost, which is comparable with the loss from guava extract alone. Neither catechol nor hydrogen peroxide had any effect on ascorbic acid when used in the absence of the guava extract.

Effect of Enzyme Extract on Ascorbic Acid: The guava enzyme extract prepared by the same method described under ''Qualitative Tests,'' was also used. Results comparable with the ones obtained when using guava extract are shown (Table 1, Columns 4 and 5).

CONCLUSIONS

The results obtained with both enzyme extract and guava extract favor the view that three enzyme systems—ascorbic acid oxidase, polyphenol oxidase, and peroxidase—are present in guava because (1) the loss in the presence of guava extract and enzyme extract alone is not increased by the addition of hydrogen peroxide, which would be expected if the system were oxidizing its naturally occurring phenolic substrates. This is evidence for the presence of ascorbic acid oxidase. (2) The addition of catechol produced an increase in the amount of ascorbic acid lost. That can take place only when an enzyme system capable of oxidizing phenolic substances is present, indicating the presence of polyphenol oxidase. (3) The addition of hydrogen peroxide in the presence of added catechol caused a marked increase in the amount of ascorbic acid lost. This result as well as those obtained when determining peroxidase activity under the action of several agents confirm the presence of peroxidase in guava tissues.

SUMMARY

The high ascorbic acid content of pulped fresh guava, when frozen, decreased fairly rapidly, one third or more of the initial (350 mg. per 100 gm.) being lost in the first month of storage. The rate was appreciably lower after this period. After four months of storage under similar conditions, the blanched, pulped guava, which originally contained 228 mg. per 100 gm., had lost only 7.5 per cent in reduced ascorbic acid.

Three enzyme systems seem to be present in guava flesh: ascorbic acid oxidase, polyphenol oxidase, and peroxidase.

Citric acid dehydrogenase and ascorbic acid dehydrogenase do not appear to be present in guava flesh.

The highest enzyme activity is located in the inner flesh and surrounding the vascular bundles. The highest ascorbic acid content is located in the skin and outer flesh.

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MICROBIOLOGY OF SPRAY-DRIED WHOLE EGG
I. PLATE AND DIRECT COUNTS [1]

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Prior to 1941 less than a half million pounds of spray-dried whole-egg powder was produced annually in the United States. Most of this went into bakery products and prepared mixes. By 1941 World War II had increased the demand for a nutritious, easily transportable egg product of good keeping quality. In that year the annual production of spray-dried whole-egg powder reached 31,000,000 pounds. In 1942 it reached 226,000,000 pounds.

Distribution of egg powder on battle fronts and among civilian populations in foreign countries soon introduced unusual handling and storage problems. Other problems arose as new ways of preparing the powder for direct consumption were devised and as the ravages of war interfered with normal kitchen practices. Powder was frequently consumed which had received little or no heat treatment. Manifestly, more attention had to be given to microbiological quality than had been given in previous years, when egg powder was used principally as an ingredient in foods which were subjected to temperatures lethal to most contaminating, vegetative-type organisms. Hence, it was not surprising that the wartime agencies charged with the manufacture, purchase, distribution, and utilization of spray-dried whole-egg powder should be interested in its microbial content and in the types of contaminants which might affect its keeping quality and wholesomeness. This type of information was not available in 1941-1942 for the reason that microbiological research in the egg-dehydration industry had not kept pace with technical developments. As a step toward the acquisition of needed information, several thousand samples of spray-dried whole-egg powder were examined by microbiological methods between September 1, 1943, and January 1, 1945. All samples represented lots of powder manufactured from unpasteurized, liquid whole egg according to United States Department of Agriculture (1943) and War Food Administration (1944) purchase specifications. They were examined for plate (viable cell) and direct microscopic (viable and dead cell) counts, *Salmonella* species, *Escherichia coli*, molds, and thermophiles. Only the plate- and direct-count findings are presented in this paper.

For the most part, the findings are presented in tables. Variations in arithmetical averages and in distributions of the plate and direct counts

are shown for each of the 16 months of the investigation; for the East
North Central, West North Central, and South Central Divisions of the
United States; and according to the age of the samples. Findings for 43
individual plants are treated briefly. Throughout the report, an effort has
been made to present the plate and direct counts in relation to the unoffi-
cial, tentative, maximum plate-count (300,000 organisms per gram) and
direct-count (10,000,000 organisms per gram) standards which the indus-
try was encouraged to meet in 1942-1944. They have also been presented
in relation to the tentative, maximum plate-count and direct-count stand-
ards (300,000 and 15,000,000, respectively) which were incorporated in
the 1945 WFA purchase specifications. This was done to facilitate com-
parisons which control bacteriologists might wish to make in evaluating
progress within the industry. It was also done because of the fact that
the 1943-1944 direct-count findings were partially responsible for the deci-
sion to increase the tentative, maximum, direct-count standard to 15,000,000
in 1945.

Samples were collected and forwarded to the laboratory by resident
government inspectors. Each sample was composited from portions of
powder intermittently collected at the time of manufacture. Each rep-
resented one to five U.S.D.A. lots (8,000- to 40,000-pound quantities).
With few exceptions, samples were examined within 24 to 72 hours of
receipt.

The procedures followed in making the plate and direct counts have
been described in a recent publication by McFarlane, Watson, and Gores-
line (1945).

RESULTS

Plate- and Direct-Count Averages, by Months: In 1943, December had
the highest plate- and direct-count averages of any of the four months
for which data were available (Table 1). The ratio of the storage shell-egg
liquid to fresh shell-egg liquid dried was also highest for this month
(Table 2).

In 1944 the monthly plate-count averages ranged from 80,000 to
1,063,000 organisms per gram. They began decreasing the first of the
year, reached a low in April, and increased slightly during May, June,
and July. There was a sharp increase in August, and the trend continued
upward through November. The lowest averages were obtained in the
March-August period. The highest average for 1944, 1,063,000 per gram,
was obtained in November. This high monthly average could not be ex-
plained by the inclusion of a few sporadic high counts in the calculation.
Examination of the individual plant findings revealed that in general
counts were proportionately higher for lots of powder manufactured in
this month. Moreover, the ratio of the storage shell-egg liquid to fresh
shell-egg liquid dried was higher in November than in any of the other
months in 1944.

No monthly direct-count averages (Table 1) were below 10,000,000. In
1944 they were below 15,000,000 in March, April, May, June, July, Sep-
tember, and December. Both February and November averages were un-
usually high. Cold-storage éggs were undoubtedly responsible for the high

November average but this explanation does not seem to account for the February average. It was noted, however, that in February 51 samples had counts greater than 50,000,000 organisms per gram and that three dehydration plants contributed approximately half of these samples. One sample had a count of 2,000,000,000; another a count of 4,192,000,000. With the exceptions of February and August, those months which had plate-count averages below 300,000 had direct-count averages below 15,-000,000. Only one month, December, 1944, had a direct-count average below 15,000,000 and a plate-count average above 300,000. Monthly direct-count averages were less regular in their seasonal trends than the monthly plate-count averages. Direct counts reached greater extremes than plate counts, and their arithmetical averages for this reason were more variable from month to month and less reflective of seasonal influences.

TABLE 1

Plate-Count and Direct-Count Averages, by Months

Month	Plate counts			Direct counts		
	Number plants contributing samples	Number samples examined	Average plate count (per gram)	Number plants contributing samples	Number samples examined	Average direct count (per gram)
1943						
September............	14	136	397,000	14	136	15,350,000
October................	29	217	570,000	29	217	21,870,000
November............	29	323	585,000	29	265	18,010,000
December............	29	215	822,000	29	215	25,960,000
1944						
January................	68	444	382,000	65	394	15,350,000
February..............	78	698	190,000	75	526	29,930,000
March....................	62	520	143,000	59	412	11,940,000
April.....................	64	531	80,000	62	489	10,190,000
May......................	67	565	89,000	67	512	14,490,000
June......................	68	567	83,000	65	527	11,070,000
July......................	63	523	93,000	63	523	11,110,000
August..................	65	605	124,000	65	605	17,060,000
September............	64	475	202,000	64	475	13,110,000
October................	59	380	513,000	59	380	16,260,000
November............	41	222	1,063,000	41	222	27,870,000
December.............	27	134	573,000	27	134	14,720,000

Bureau of Agricultural Economics figures for the production of dried eggs from the different sources of liquid egg, by months, from September 1943 to December 1944, inclusive, are given (Table 2). A comparison of these data with those in Table 1 reveals that the monthly average counts varied with the sources of the liquid egg, that is, the averages decreased as the proportions of fresh shell-egg liquid to frozen-egg and storage shell-egg liquids dried each month increased. In April, 98 per cent of the powder was manufactured from fresh shell-egg liquid and only two per cent from frozen-egg and storage shell-egg liquids. April had the lowest plate- and direct-count averages. Starting in May, the monthly average counts began to go up as the proportions of the monthly powder productions from frozen-egg and storage shell-egg liquids began to increase, and as the

proportions from fresh shell-egg liquid began to decrease. In months in which the counts were high, such as in October, November, and December 1944, 60 to 80 per cent of the powder was manufactured from frozen-egg and storage shell-egg liquids. The relationship between the monthly average counts and the sources of the liquid egg dried was more regularly

TABLE 2

Production of Dried Whole Egg, by Months [1]

Month	From fresh shell-egg liquid	From frozen-egg liquid	From storage shell-egg liquid	Total liquid dried
1943				
September	7,373 [2]	7,432	4,857	19,662
	(37.5%)	(37.8%)	(24.7%)	
October	4,496	8,717	9,847	23,060
	(19.5%)	(37.8%)	(42.7%)	
November	3,783	9,986	8,226	21,995
	(17.2%)	(45.4%)	(37.4%)	
December	1,926	13,273	5,736	20,935
	(9.2%)	(63.3%)	(27.4%)	
1944				
January	12,845	5,994	2,569	21,408
	(60.0%)	(28.0%)	(12.0%)	
February	24,062	629	478	25,169
	(95.6%)	(2.5%)	(1.9%)	
March	29,570	576	151	30,297
	(97.6%)	(1.9%)	(0.5%)	
April	29,730	545	30	30,305
	(98.1%)	(1.8%)	(0.1%)	
May	32,076	396	562	33,034
	(97.1%)	(1.2%)	(1.7%)	
June	29,624	602	1,457	31,683
	(93.5%)	(1.9%)	(4.6%)	
July	24,570	2,964	3,025	30,559
	(80.4%)	(9.7%)	(9.9%)	
August	23,134	6,019	4,472	33,625
	(68.8%)	(17.9%)	(13.3%)	
September	12,024	7,958	4,657	24,639
	(48.8%)	(32.3%)	(17.9%)	
October	9,453	8,062	6,058	23,573
	(40.1%)	(34.2%)	(25.7%)	
November	5,514	6,478	4,617	16,609
	(33.2%)	(39.0%)	(27.8%)	
December	2,104	7,138	1,225	10,467
	(20.1%)	(68.2%)	(11.7%)	
Total	252,284	86,769	57,967	397,020
	(63.5%)	(21.9%)	(14.6%)	

[1] Bureau of Agricultural Economics production figures.   [2] Figures given in pounds.

reflected by the plate-count averages than by the direct-count averages.

Plate- and Direct-Count Frequency Distributions, by Months: Plate counts were made on 6,555 samples. These were received from 101 dehydration plants located in 26 states. Plate counts ranged from less than 1,000 to 105,000,000 organisms per gram. Approximately 57 per cent of the samples had counts less than 100,000 per gram, 81 per cent less than

300,000, and 88 per cent less than 500,000. Breakdowns of the samples on a monthly basis according to the numbers and percentages which had counts less than ($<$) and greater than ($>$) 100,000, 300,000, and 500,000 are shown (Table 3). Values of 100,000 and 500,000 were introduced for purposes of comparison with the tentative, maximum plate count of 300,000 microorganisms per gram because they had been discussed at one time or another as possible standards. The Canadian total viable-count standard for Grade A powder was 500,000 per gram, Johns (1944) and Special Products Board (1944). In the Canadian procedure the plates were incubated for 48 hours at 37°C.(98.6°F.), whereas in the procedure followed in this study the plates were incubated for 72 hours at 30°C.(86°F.). As may be noted (Table 3), higher percentages of acceptable samples were manufactured during March, April, May, June, July, and August than during the remaining six months of the year. This held true whether 100,000, 300,000, or 500,000 organisms per gram was considered the desirable maximum count. Furthermore, a greater number of dehydration plants were able to produce lots of powder with monthly average plate counts under 300,000 per gram in the March-August period.

The monthly percentages (Table 3) show a seasonal relationship which rather closely parallels that for egg production; that is, more acceptable lots of powder, based on plate counts, were produced in months of high egg production. Bureau of Agricultural Economics egg-production figures for each month of the investigation are given below for comparison.

PRODUCTION OF INDIVIDUAL SHELL EGGS, BY MONTHS

1943		1944					
millions		*millions*		*millions*		*millions*	
Sept 3,336	Jan. 4,492	May 6,744	Sept. 3,533
Oct. 3,012	Feb. 5,407	June 5,472	Oct. 3,292
Nov. 2,747	Mar. 6,829	July 4,668	Nov. 3,006
Dec. 3,295	Apr. 7,040	Aug. 4,035	Dec. 3,412

Direct counts were made on 6,032 of the samples. They ranged from less than 1,000,000 to 4,192,000,000 organisms per gram. Approximately 75 per cent of the direct counts were less than 10,000,000, per gram and 83 per cent less than 15,000,000. Breakdowns of the samples on a monthly basis according to the numbers and percentages which had counts less than and greater than 10,000,000 and 15,000,000 are shown (Table 4). More acceptable lots of powder based on direct counts were produced in months of high shell-egg production. However, as expressed in terms of monthly productions, the percentages of samples meeting the direct-count standards (Table 4) were less regular in their seasonal trends than the percentages of samples meeting the plate-count standards (Table 3).

Distribution ranges of the counts by months are shown (Tables 5 and 6); the particular ranges were selected for the purpose of demonstrating some of the more salient features of the gross distributions. The number of samples (counts) falling within each range is enumerated. The data as arranged in each table simulate a graphic display of the seasonal variations in the microbial counts in egg powder. Large numbers of samples had counts in the lowest distribution ranges in those months in which the highest

TABLE 3

Monthly Incidence of Samples Which Had Plate Counts Less Than and Greater Than

100,000, 300,000 and 500,000

Month	Number samples examined	Samples which had plate counts										
		<100,000		>100,000		<300,000		>300,000		<500,000		>500,000
			pct.		pct.		pct.		pct.		pct.		pct.
1943													
Sept.	136	32	23.5	104	76.5	90	66.2	46	33.8	108	79.4	28	20.6
Oct.	217	22	10.1	195	89.9	105	48.4	112	51.6	133	61.3	84	38.7
Nov.	323	52	16.1	271	83.9	166	51.4	157	48.6	222	68.7	101	31.3
Dec.	215	16	7.4	199	92.6	88	40.9	127	59.1	121	56.3	94	43.7
1944													
Jan.	444	193	43.5	251	56.5	298	67.1	146	32.9	349	78.6	95	21.4
Feb.	698	466	66.8	232	33.2	593	85.0	105	15.0	646	92.6	52	7.4
March.	520	390	75.0	130	25.0	458	88.1	62	11.9	486	93.5	34	6.5
April.	531	428	80.6	103	19.4	503	94.7	28	5.3	517	97.4	14	2.6
May.	565	454	80.4	111	19.6	534	94.5	31	5.5	549	97.2	16	2.8
June.	567	447	78.8	120	21.2	546	96.3	21	3.7	554	97.7	13	2.3
July.	523	411	78.6	112	21.4	512	97.9	11	2.1	514	98.3	9	1.7
Aug.	605	410	67.8	195	32.2	574	94.9	31	5.1	591	97.7	14	2.3
Sept.	475	233	49.1	242	50.9	402	84.6	73	15.4	437	92.0	38	8.0
Oct.	380	104	27.4	276	72.6	269	70.8	111	29.2	307	80.8	73	19.2
Nov.	222	36	16.2	186	83.8	99	44.6	123	55.4	148	66.7	74	33.3
Dec.	134	31	23.1	103	76.9	65	48.5	69	51.5	78	58.2	56	41.8
Total	6,555	3,725	56.8	2,830	43.2	5,302	80.9	1,253	19.1	5,760	87.9	795	12.1

percentages of samples met the tentative standards. As noted before, these were months of high shell-egg production. They were also the months in which the far greater proportion of powder production was from fresh shell-egg liquid.

Plate- and Direct-Count Frequency Distribution, by Plants: Distribution ranges of the plate and direct counts are given (Tables 7 and 8) for 43 plants which forwarded 50 or more samples. Considering the innumerable variations in the structure and operation of spray driers, it is not surprising that there was a variety of distribution patterns. Each plant displayed characteristic individuality.

A few plants produced egg powders of uniformly good, microbial quality from the standpoint of the unofficial plate- and direct-count standards; for example, plate counts on 88 samples received from plant No. 1 ranged from 1,000 to 289,000 with 10 above 100,000. Seven of the 10 plate counts which exceeded 100,000 were obtained on samples manufactured during the first two months of the study. Direct counts, made on 72 of the 88 samples, ranged from less than 1,000,000 to 12,000,000, with only one above 10,000,000. Plate counts on the 96 samples received from plant No. 29 ranged from 7,000 to 264,000, with six above 100,000. The direct counts on these same samples ranged from less than 1,000,000 to 14,000,000, with only four above 10,000,000.

Some plants, illustrated by Nos. 4, 7, 17, 20, 24, 30, 35, and 38, rather consistently produced lots of high-count powder. Others which started out in 1943 or in the early part of 1944 to produce reasonably low-count powders produced high-count powders during the latter part of the 1944 season. High-count powders produced in the latter months of 1944 by Plants 11, 12, 37, 42, and others cannot be entirely explained by conditions beyond the manufacturers' control, which existed in the industry at that time.

In spite of wartime handicaps, there were dehydration plants which exercised care in the selection of eggs and which followed efficient and sanitary operating practices. These plants produced high percentages of samples which met both the plate- and the direct-count, unofficial, tentative standards of 1942-1944. Ninety-four to 100 per cent of the samples from Plants 1, 10, 15, 29, and 36 and 81 to 89 per cent of samples from Plants 6, 13, 21, 22, 32, 39, 40, 41, and 43 met both standards. In contrast, only 9 to 30 per cent of the samples examined from Plants 4, 7, 17, 20, 24, 30, 35, and 38 met standards. These eight plants are recorded above as having consistently produced lots of high-count powder. It is interesting that neither the "good" nor the "poor" plants were grouped in a limited area. The 14 "good" plants were located in nine midwestern states, including the southern and northern states of Texas and Minnesota; the eight "poor" plants were in seven states. In three instances "good" and "poor" plants operated in the same states. Plants 40 (good) and 20 (poor) were in the same city.

No attempt was made to determine whether the monthly plate- and direct-count distribution ranges for individual plants reflected a seasonal influence, mainly because of the small numbers of samples examined per plant. Monthly average counts calculated for Plants 13, 17, 21, 22, 24, 25,

TABLE 4

Monthly Incidence of Samples Which Had Direct Counts Less Than and Greater Than 10,000,000 and 15,000,000

Month	Number samples examined	Samples which had direct counts							
		<10,000,000		>10,000,000		<15,000,000		>15,000,000	
1943			*pct.*		*pct.*		*pct.*		*pct.*
Sept............	136	100	73.5	36	26.5	112	82.4	24	17.6
Oct.............	217	122	56.2	95	43.8	148	68.2	69	31.8
Nov............	265	187	70.6	78	29.4	199	75.1	66	24.9
Dec............	215	125	58.1	90	41.9	138	64.3	77	35.7
1944									
Jan.............	394	306	77.7	88	22.3	327	83.0	67	17.0
Feb.............	526	410	77.9	116	22.1	429	81.6	97	18.4
March.........	412	352	85.4	60	14.6	366	88.8	46	11.2
April..........	489	422	86.3	67	13.7	433	88.5	56	11.5
May............	512	428	83.6	84	16.4	447	87.3	65	12.7
June...........	527	385	73.1	142	26.9	443	84.1	84	15.9
July............	523	432	82.6	91	17.4	467	89.3	56	10.7
Aug............	605	427	70.6	178	29.4	498	82.3	107	17.7
Sept............	475	341	71.8	134	28.2	397	83.6	78	16.4
Oct.............	380	260	68.4	120	31.6	319	84.0	61	16.0
Nov............	222	132	59.5	90	40.5	162	73.0	60	27.0
Dec............	134	80	59.7	54	40.3	98	73.1	36	26.9
Total........	6,032	4,509	74.8	1,523	25.2	4,983	82.6	1,049	17.4

TABLE 5

Plate-Count Distribution Ranges, by Months

Month	Number of counts falling within the range							
	0-50,000	50,001-100,000	100,001-200,000	200,001-300,000	300,001-400,000	400,001-500,000	500,001-1,000,000	1,000,001-over
1943								
September.............	9	23	38	20	14	4	19	9
October................	8	14	44	39	11	17	53	31
November.............	20	32	52	62	35	21	68	33
December.............	5	11	36	36	19	14	46	48
1944								
January................	141	52	80	25	34	17	44	51
February..............	335	131	95	32	37	16	34	18
March..................	298	92	55	13	15	13	22	12
April...................	349	79	56	19	7	7	11	3
May.....................	370	84	55	25	8	7	11	5
June....................	319	128	74	25	8	0	10	3
July.....................	239	172	86	15	2	0	6	3
August.................	218	192	139	25	11	6	6	8
September.............	82	151	127	42	20	15	23	15
October................	42	62	109	56	24	14	38	35
November.............	13	23	37	26	25	24	43	31
December.............	18	13	25	9	6	7	24	32
Total..........	2,466	1,259	1,108	469	276	182	458	337

TABLE 6

Direct-Count Distribution Ranges, by Months

Month	Number of counts falling within the range												
	0-5,000,000	5,000,001-10,000,000	10,000,001-15,000,000	15,000,001-20,000,000	20,000,001-30,000,000	30,000,001-40,000,000	40,000,001-50,000,000	50,000,001-60,000,000	60,000,001-70,000,000	70,000,001-80,000,000	80,000,001-90,000,000	90,000,001-100,000,000	100,000,001-OVER
1943													
September	43	57	12	6	2	3	1	1	1	2	3	1	4
October	42	80	26	17	16	5	5	4	4	3	2	1	12
November	123	64	12	10	7	7	11	7	4	4	4	3	9
December	101	24	13	9	14	11	3	4	7	6	4	3	16
1944													
January	246	60	21	9	13	5	8	3	4	2	3	5	15
February	348	62	19	17	15	10	4	5	6	3	5	4	28
March	305	47	14	5	5	4	8	2	4	4	2	2	12
April	379	43	11	11	14	9	5	1	3	6	0	0	7
May	336	92	19	11	17	5	7	3	2	1	2	2	15
June	233	152	58	25	27	9	5	3	3	2	2	3	5
July	265	167	35	15	10	4	9	3	2	3	1	1	8
August	204	223	71	22	30	22	5	2	5	3	0	1	17
September	150	191	56	13	22	16	5	1	5	6	1	2	7
October	135	125	59	15	10	7	4	3	5	3	4	1	9
November	58	74	30	19	13	4	7	3	2	1	2	0	9
December	46	34	18	7	12	5	3	2	3	2	1	0	1
Total	3,014	1,495	474	211	227	126	90	47	60	49	36	29	174

TABLE 7

Plate-Count Distribution Ranges, by Plants

Plant No.	Number of counts falling within the range								Number of samples examined
	0-50,000	50,001-100,000	100,001-200,000	200,001-300,000	300,001-400,000	400,001-500,000	500,001-1,000,000	1,000,001-over	
1.............	65	13	7	3	0	0	0	0	88
2.............	56	51	49	11	4	8	20	11	210
3.............	17	19	16	5	1	2	1	1	62
4.............	2	3	3	8	5	4	5	20	50
5.............	20	18	11	4	3	2	13	15	86
6.............	88	69	56	28	13	3	1	0	258
7.............	9	12	4	3	4	6	13	1	52
8.............	22	10	20	3	0	1	0	3	59
9.............	30	11	6	4	5	2	2	0	60
10.............	60	9	3	0	0	0	0	0	72
11.............	7	11	26	9	5	3	15	15	91
12.............	10	7	12	5	5	3	14	9	65
13.............	325	129	72	37	22	11	11	4	611
14.............	28	22	28	15	9	6	8	5	121
15.............	58	19	2	0	0	0	0	0	79
16.............	14	17	31	9	3	5	10	4	93
17.............	7	18	23	30	26	24	55	23	206
18.............	111	21	14	5	0	1	2	1	155
19.............	18	17	28	10	7	2	5	0	87
20.............	8	12	8	7	5	1	7	26	74
21.............	105	24	37	26	13	7	7	0	219
22.............	89	28	39	4	7	4	7	0	178
23.............	34	19	13	7	6	7	16	18	120
24.............	17	24	48	25	17	14	45	52	242
25.............	82	59	15	14	9	6	26	25	236
26.............	36	21	5	4	2	1	3	8	80
27.............	22	41	56	32	14	6	8	0	179
28.............	34	36	44	7	3	2	5	2	133
29.............	60	30	4	2	0	0	0	0	96
30.............	14	20	17	12	6	7	32	17	125
31.............	18	14	12	9	3	2	6	2	66
32.............	46	22	14	7	5	0	3	2	99
33.............	32	7	8	2	0	2	2	2	55
34.............	58	8	13	5	3	1	2	1	91
35.............	1	2	9	7	6	6	21	2	54
36.............	28	15	11	1	0	0	0	0	55
37.............	52	38	37	12	3	4	14	6	166
38.............	6	15	16	7	7	2	4	0	57
39.............	25	16	20	10	1	0	0	0	72
40.............	51	6	11	4	1	1	1	0	75
41.............	41	6	19	2	1	0	1	0	70
42.............	34	21	7	5	0	4	3	13	87
43.............	52	10	21	3	3	0	7	2	98

Direct-Count Distribution Ranges, by Plants

Plant No.	Number of counts falling within the range													Number of samples examined
	0-5,000,000	5,000,001-10,000,000	10,000,001-15,000,000	15,000,001-20,000,000	20,000,001-30,000,000	30,000,001-40,000,000	40,000,001-50,000,000	50,000,001-60,000,000	60,000,001-70,000,000	70,000,001-80,000,000	80,000,001-90,000,000	90,000,001-100,000,000	100,000,001-over	
1	55	16	1	0	0	0	0	0	0	0	0	0	0	72
2	156	39	8	2	2	1	0	0	1	0	0	0	1	210
3	17	26	6	1	2	1	0	1	0	1	0	0	1	55
4	3	11	5	2	5	5	6	1	0	1	1	0	1	41
5	33	39	7	0	2	0	0	0	0	0	0	0	0	81
6	163	75	12	4	3	1	0	0	0	0	0	0	0	258
7	4	19	11	4	3	1	0	0	1	1	0	0	3	46
8	17	14	10	2	5	3	0	0	0	0	0	0	1	52
9	18	18	9	1	2	2	1	0	0	0	0	0	2	53
10	60		1	0	0	0	0	0	0	0	0	0	0	61
11	25	32	19	4	3	4	2	1	1	0	0	0	0	91
12	4	30	13	1	9	6	0	0	0	1	1	0	0	65
13	429	143	22	8	6	3	0	0	0	0	1	0	0	611
14	28	63	20	7	2	0	0	1	1	0	0	0	0	121
15	59	6	0	0	0	0	0	0	0	0	0	0	0	65
16	41	22	4	7	2	0	0	0	0	0	0	0	0	76
17	11	20	12	10	17	14	18	10	17	14	10	12	41	206
18	85	34	14	7	8	2	2	1	1	1	0	0	1	155
19	18	12	13	8	10	6	1	1	0	1	0	0	1	71
20	13	9	5	1	3	3	5	0	3	2	3	0	17	64
21	152	61	6	0	0	0	0	0	0	0	0	0	0	219
22	99	62	13	3	1	0	0	0	0	0	0	0	0	178
23	56	37	11	5	6	4	1	0	0	0	0	0	0	120
24	14	25	25	30	38	24	19	9	16	10	6	8	18	242
25	66	63	31	13	10	9	6	5	4	4	4	0	18	234
26	33	12	7	2	0	3	0	1	0	0	0	0	1	64
27	103	55	15	3	3	0	0	0	0	0	0	0	0	178
28	61	36	11	5	1	1	0	0	0	0	0	0	0	115
29	82	10	4	0	1	0	0	0	0	0	0	0	0	96
30	12	20	7	5	0	5	12	8	7	6	0	5	19	124
31	16	20	7	6	14	2	2	1	0	0	0	0	0	56
32	76	19	3	1	2	0	0	0	0	0	0	0	0	99
33	22	13	5	1	0	0	1	0	0	0	1	0	3	45
34	52	16	6	5	2	3	0	0	0	1	0	0	0	91
35	16	17	6	1	5	1	0	0	1	0	0	0	1	43
36	38	9	2	1	1	0	0	4	2	0	1	1	16	50
37	71	62	13	7	6	1	0	0	0	0	0	0	0	166
38	5	4	9	7	1	1	2	0	0	1	0	0	0	49
39	37	8	2	3	1	1	0	0	0	0	1	0	1	52
40	38	16	5	1	1	0	0	0	2	0	0	0	5	61
41	45	11	3	0	2	0	1	0	0	0	0	0	0	62
42	27	32	11	4	3	2	0	0	0	0	0	0	5	87
43	63	27	6	1	0	0	0	1	0	0	0	0	0	98

27, and 37 displayed differences corresponding in order and degree to those displayed in Table 1.

Plate- and Direct-Count Averages and Distributions, by U. S. Divisions: Samples from the South Central Division had the lowest plate- and direct-count averages; samples from the East North Central had the highest (Table 9). By the same token, more samples from the South Central Division than from either of the other two divisions met the tentative plate- and direct-count standards (Table 10). Plate- and direct-count averages and distributions were given only for the three midwestern divisions because it was felt that the numbers of samples examined from other U. S. divisions were too few to be representative of their respective productions.

TABLE 9

Average Plate and Direct Counts on Samples From East North Central, West North Central, and South Central Divisions of the United States

Division	Plate			Direct		
	Number of plants contributing samples	Number of samples examined	Average plate count (per gram)	Number of plants contributing samples	Number of samples examined	Average direct count (per gram)
East North Central (Ohio, Ind., Ill., Mich., and Wis.).........	26	1,726	412,000	26	1,569	29,862,000
West North Central (Minn., Iowa, Mo., N. Dak., S. Dak., Nebr., and Kans.)......	40	1,928	251,000	39	1,684	16,568,000
South Central (Ky., Tenn., Ala., Miss., Ark., La., Okla.. and Tex.).........	25	2.324	182.000	25	2.215	7.862.000

TABLE 10

Distribution of Plate and Direct Counts on Samples From East North Central, West North Central, and South Central Divisions of the United States [1]

Division	Samples which had					
	Plate counts		Direct counts			
	< 300,000		< 10,000,000		< 15,000,000	
		pct.		pct.		pct.
East North Central.................	1,169	67.7	953	60.7	1,088	69.3
West North Central.................	1,609	83.5	1,226	72.8	1,350	80.2
South Central.........................	1,968	84.7	1,880	84.9	2,053	92.7

[1] Plate and direct counts are considered independently.

Plate-Count Averages and Distributions of Samples According to Age: Arithmetical averages of the plate counts were computed for samples of the same age in days. The age of each sample was calculated from the date of manufacture (or, if composited from more than one U.S.D.A. lot, from the date of manufacture of the last lot included) to the day of examination. Because of insufficient information, the age of every sample could not be determined. Samples of known age ranged from one to 43 days.

There was a trend (Table 11) for the samples in the older age groups to have the lower average plate counts, and for the age groups which had the lower average counts to contain the higher percentages of samples which met the tentative plate-count standard. The tabular data also indicate that the rate of destruction of viable organisms in the samples might have been greatest during the first few days of the holding period. These findings are similar to those which might be expected in a routine powder-storage study of the same duration. They are in line with data published by Gibbons and Fulton (1943), Johns (1944), and Stuart, Goresline, Smart, and Dawson (1945) for the higher holding temperatures. It should be remembered, however, that in this study the samples of the same age may have been manufactured in several different plants and/or at different times of the year. This may well explain why the samples of a successive age period did not always show the anticipated decrease in average plate count. For example, the 660 nine-day-old samples had an average plate count of 191,000 per gram; the 558 10-day-old samples had an average of 297,000. Some of the age groups contained very few samples. Only 158 18- to 43-day-old samples were examined. They had an average plate count of 141,000 per gram. Ninety-two per cent had plate counts less than 300,000.

TABLE 11

Plate-Count Averages and Distributions of Samples According to Age

Age	Number of samples of same age	Average plate count (per gram)	Samples which had plate counts <300,000
days			*pct.*
2	10	1,091,000	......
3	61	678,000	67.2
4	213	999,000	65.7
5	510	388,000	71.8
6	878	310,000	78.6
7	1013	237,000	80.7
8	806	202,000	82.6
9	660	191,000	83.9
10	558	297,000	82.6
11	462	146,000	90.3
12	310	141,000	86.8
13	226	232,000	83.6
14	124	130,000	87.9
15	67	131,000	88.1
16	65	213,000	84.6
17	61	136,000	86.9

·DISCUSSION

It was believed that this investigation would reveal seasonal differences in the degree of contamination of spray-dried whole-egg powder. Colder seasonal weather should cause a reduction in the rate of multiplication of bacterial cells in shell and liquid eggs and a reduction in the rate of death in shell, liquid, and dried eggs. This action might bring about an increase in the ratio of viable to total (viable and dead) cells in these products. Plate- and direct-count monthly averages would be expected to

increase as the proportions of the frozen and cold-storage shell-egg liquids to the fresh shell-egg liquid dehydrated each month increase. The reasonableness of these expectations for the conditions of manufacture is supported by the data (Tables 1, 2, 3, 4, 5, and 6).

The markedly increased microbial contamination apparent in the fall and winter of 1943-1944 may be principally attributed to the quality of the eggs available for dehydration. It is known that all shell eggs were not properly handled during the peak laying seasons in 1943-1944 because of increased production, shortage of egg cases, delayed transportation, and scarcity of labor. Moreover, cold-storage and freezing facilities were inadequate for establishing backlogs of high-quality storage and frozen eggs for fall and winter drying. Although sanitary practices were being progressively improved in the egg-dehydration industry, there were other factors closely associated with the manufacture of powder which affected the over-all plate- and direct-count picture during the period of the investigation. Various types of driers—Barnhill, Douthitt, Rogers, Majonnier, and modifications of these—were in operation. Some were less efficient than others in lowering the microbial content of the egg substance. Processing procedures were being continually changed in an endeavor to improve the quality of the powder and the efficiency of operation and to increase production. Shortages existed in equipment and in cold-storage space for packaged powder.

A preliminary survey of the plate- and direct-count findings in 1944 disclosed that more samples were able to meet a maximum plate-count standard of 300,000 organisms per gram than a maximum, direct-count standard of 10,000,000. This suggested that the unofficial standards for the two counts might be out of line and that a higher maximum, direct-count standard might enable a fairer evaluation of the samples. It had been assumed that some degree of correlation ought to exist between the two types of counts. This assumption seemed reasonable because processing methods were essentially the same, the liquid egg was not subjected to pasteurization temperatures, and the moisture content was not reduced below the four- to six-per cent range. Eighty-one per cent of the plate counts were less than 300,000 organisms per gram and 83 per cent of the direct counts were less than 15,000,000 (Tables 3 and 4). These percentages, however, were obtained by an independent consideration of each type of count. Eighty-one to 83 per cent of the samples did not meet desirable plate- and direct-count requirements when the findings were evaluated in terms of each individual sample's ability to meet a plate count of 300,000 per gram and a direct microscopic count of 10,000,000 or 15,000,000 per gram. This is shown in the following tabulation, which lists the percentages of samples that had counts less than and greater than tentative, maximum standard counts. Only 68 per cent of the 6,032 samples on which both plate and direct counts were made met both the plate- and direct-count, unofficial, tentative standards proposed in 1942-1944. Seventy-three per cent could have met the tentative standards supported in 1945.

Plate counts	Direct counts		Direct counts	
(1942-1944, 1945 maximum)	(1942-1944 maximum)		(1945 maximum)	
	<10,000,000	>10,000,000	<15,000,000	>15,000,000
<300,000	68%	13%	73%	8%
>300,000	7%	12%	10%	9%

The tabulated percentages show a gross correlation between plate and direct counts for approximately 80 per cent of the samples, that is, if the 68 per cent which had both type counts below the 1942-1944 standards are combined with the 12 per cent which had both type counts above. Had the 1945 direct-count standard been in effect, the correlation between the two types of counts would have held for 82 per cent of the samples. These observations should not be interpreted by the control bacteriologist to mean that 80 per cent or more of any given number of spray-dried whole-egg samples will necessarily show a direct correlation between the plate and direct counts. Too many factors are involved to draw any such conclusion. Johns and Berard (1944, 1945) have already pointed out the effect of heat on the direct count. Therefore, it cannot be anticipated that samples manufactured by other processing methods, for example, by those employing heat treatment of the liquid egg, will yield data comparable to the above.

Regional differences in the degree of microbial contamination in spray-dried whole-egg powder could not be accurately evaluated. For one reason, the interregional distribution of fresh, frozen, and storage eggs for dehydration was commonplace during the period of the investigation. For another reason, the United States divisions were not representative of distinctly different soils and climates. However, processing difficulties were expected in the South because of the higher temperatures. Southern dehydration plants were more frequently inspected and more rigidly supervised. These actions may explain why the South Central Division had a higher percentage of samples which met the unofficial, tentative, plate- and direct-count standards in 1942-1944.

The inference that samples from the South Central Division may have had proportionately lower counts because they had farther to travel to the laboratory (Philadelphia) and hence were older at the time of their examination is not supported by the data. There were no appreciable differences in the mean ages of the samples from the three divisions—E.N.C., 8.8 days; W.N.C., 8.1 days; and S.C., 9.1 days. In no instance were all the samples from any one of the 43 plants (Tables 7 and 8) of the same age at the time of examination. However, most of the samples from a given plant fell within two days plus or minus the mean age for the plant. There were exceptions. Wartime delays in mailing, transportation, and delivery were more instrumental than distance in determining the respective ages of the samples.

SUMMARY

Plate- (viable cell) and direct-microscopic-count findings on more than 6,000 samples of spray-dried whole egg have been presented as a partial record of the microbial quality of egg powder manufactured in the

United States between September 1, 1943, and January 1, 1945. The samples represented lots of powder manufactured according to United States Department of Agriculture and War Food Administration purchase specifications.

In general, proportionately more lots of low microbial-count powders and lots with lower monthly averages were produced in the March-August period. The six months in this period were months of high shell-egg production. They were also months in which the far greater part of the powder was manufactured from fresh shell-egg liquid.

Counts varied with the kind of liquid egg used for drying. Monthly averages became progressively lower as increasingly larger quantities of fresh shell-egg liquid and decreasing quantities of frozen-egg and storage shell-egg liquids were dried. This trend was more regularly reflected by the monthly plate-count averages.

When the two kinds of counts were considered independently, 81 per cent of the samples examined had plate counts less than 300,000 organisms per gram (unofficial, maximum, plate-count standard), and 75 per cent had direct counts less than 10,000,000 organisms per gram (unofficial, maximum, direct-count standard). Only 68 per cent of the samples on which both kinds of counts were made met both standards.

Individual dehydration plants varied in ability to produce powder of the desired microbial quality. Whereas 81 to 100 per cent of the egg-powder samples from 14 of the better egg-dehydration plants met both the plate- and the direct-count standards. cited above, only nine to 30 per cent of the samples from eight of the poorer plants met them.

Samples in older age groups (grouped according to age in days at the time of examination) had lower plate-count averages, and higher percentages of these samples met the plate-count standard.

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SODIUM AND POTASSIUM CONTENT OF MEATS

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The necessity for a rapid and accurate method for the determination of sodium and potassium has led to the development of the flame photometer. The photometer was devised by Barnes, Richardson, Berry, and Hood (1945). This instrument involves the use of the common flame test and the photoelectric cell.

The material to be tested is ashed and taken up with a small quantity of acid, made up to volume, and then introduced into an atomizer. The spray is carried to the flame by way of the air vent of the burner. The emitted light characteristic of the element is isolated by the proper filter, focused on a photoelectric cell which gives rise to a current which is proportional to the concentration of the element sought.

EXPERIMENTAL PROCEDURE

For this experiment there were 71 samples of meat including several each of lean beef, medium cut beef, beef liver, beef kidney, veal, and lamb. One beef-brain sample was included. The preliminary treatment of these samples is described by Toscani (1945).

For analysis two grams of the dried-meat sample were placed in a Vycor crucible and two c.c. of 4N sulphuric acid were added. The crucible was placed on a warm hot plate or steam bath and heating was continued until the material was carbonized. The crucible was then placed in a muffle furnace and heated for six to eight hours at 500 to 550°C.(932 to 1022°F.) or until ashing was complete.

The ash was taken up with two c.c. of concentrated hydrochloric acid and transferred to a 100-c.c. volumetric flask and made up to volume. This ash solution was diluted 1:1 for the sodium determination and 1:5 for the potassium estimation, as the latter element appears in greater quantities in meats. The diluted ash solutions were then ready for the photometer.

Berry, Chappell, and Barnes (1946) have shown that the presence of various mineral acids contained in the ash solutions introduce appreciable errors in the determination of unknown solutions. This interference is overcome by preparing standards containing the same acid and having the same concentrations as the unknown solution.

STANDARDIZATION OF THE PHOTOMETER

The calibration curves for the sodium and potassium are valid only when the pressure of the air and gas are kept constant. The air pressure for the atomizer is maintained at 10 pounds per square inch, while the pressure of the propane gas is maintained at 2.5 pounds per square inch. The proper filter is switched into place, the burner is lighted, and the

pressures of air and gas are adjusted. Distilled water is introduced into the atomizer through a small funnel and the galvanometer reading is set at zero. A solution containing 100 parts per million of the element sought is now delivered into the atomizer and the galvanometer is set at 100. When the zero reading for water and the 100 reading for the salt is constant, a known solution containing 50 parts per million of the element is placed in the atomizer and the reading recorded. This procedure is repeated using 25 parts per million and the curve is established for that element.

For accuracy, after several unknown determinations it is advisable to recheck the curve by using the standard solutions containing 50 and 100 parts per million, respectively.

DISCUSSION

There were 19 samples of well-trimmed beef muscle, 10 of untrimmed beef muscle, 19 of beef tongue, 13 of beef liver, two of beef kidney, one of beef brain, four of lamb muscle, and three of veal muscle. The recovery of added salts to the meat sample is given (Tables 1 and 2); the sodium

TABLE 1

Recovery of Added Sodium

In meat	Added	Found	Recovered	Recovery
$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	*pct.*
6.8	10.0	16.0	9.2	92.0
6.8	20.0	26.5	19.7	98.5
6.8	30.0	35.0	28.2	94.0

TABLE 2

Recovery of Added Potassium

In meat	Added	Found	Recovered	Recovery
$\mu g.$	$\mu g.$	$\mu g.$	$\mu g.$	*pct.*
9.0	10.0	19.0	10.0	100.0
9.0	20.0	28.0	19.0	95.0
9.0	30.0	37.5	28.5	95.0

and potassium contents of the various tissues and the ranges into which the samples fall, as well as values for maximum, minimum, and average content and the mean deviation of the various cuts of meat are shown (Table 3).

The average values found showed close agreement with the values given by McCance and Widdowson (1940) on the following specimens: sodium in lean beef, beef brain, tongue, and lamb; potassium in lean beef, medium beef, liver, and veal. Potassium showed a higher content in tongue, while lower potassium values were found in liver, lamb muscle, and veal. One sample of lean beef gave a value of 0.527 for potassium on repeated analyses. Beef liver gave the widest variation in the sodium content.

Bassett, Elden, and McCann (1931) analyzed 10 duplicate diets and found that the average sodium values were 18.6 per cent above those calculated from Sherman (1932) tables. They suggest that it is quite possible that the geographical area from which the food supplies came determines the magnitude of the differences.

TABLE 3

Sodium and Potassium per 100 Grams of Meat, Moist Weight
Range of Figures

Nature of meat	Sodium		Potassium	
	Number of samples	Range	Number of samples	Range
		gm.		*gm.*
Beef muscle, well trimmed	10	.047 - .060	3	.262 - .280
	5	.061 - .073	10	.289 - .310
	2	.084 - .090	5	.315 - .331
	2	.123 - .167	1	.527 ......
		Av. .070		Av. .313
		M.D. .007		M.D. .013
Beef muscle, not trimmed	5	.068 - .096	3	.297 - .320
	3	.110 - .118	5	.330 - .362
	2	.146 - .167	2	.375 - .405
		Av. .107		Av. .345
		M.D. .009		M.D. .010
Beef tongue	5	.050 - .060	2	.134 - .141
	5	.064 - .070	10	.169 - .190
	4	.071 - .080	6	.193 - .225
	5	.081 - .093	1	.262 ......
		Av. .071		Av. .189
		M.D. .003		M.D. .006
Beef liver	3	.053 - .083	1	.227 ......
	5	.097 - .154	6	.263 - .290
	4	.175 - .236	5	.296 - .320
	1	.346	1	.394 ......
		Av. .155		Av. .292
		M.D. .023		M.D. .010
Beef kidney	2	.147 - .172	2	.226 - .241
		Av. .159		Av. .234
Beef brain	1	.... .155	1	.... .265
Lamb muscle	4	.079 - .140	4	.204 - .295
		Av. .097		.... .249
Veal muscle	3	.115 - .196	3	.267 - .354
		Av. .156		Av. .301

The large individual variation of the sodium content reveals the necessity for analyzing diets fed to subjects on metabolic studies.

CONCLUSIONS

The sodium and potassium content of 71 samples of various cuts of meat was determined by flame photometry. The individual values for sodium show wide variations, while the average values show agreement with the published data.

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LIFTING POWER OF DRIED WHOLE EGG WHEN USED IN SPONGE CAKE [1]

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Although it was soon established that the nutritive value of the proteins of spray-dried egg powder was not impaired by the drying procedure, the acceptability of the first dried-egg powders, produced for lend-lease and the overseas armed forces, from the standpoint of palatability was low and decreased rapidly with storage. In addition, the dried-egg powder did not perform functionally like fresh egg. Its thickening and aerating power in products such as custards and sponge cakes decreased rapidly with aging of the powder. The palatability of the spray-dried egg powder, its keeping quality, and its culinary quality were improved during the war by various means, such as better supervision of the drying process, drying to a lower moisture content, gas packing, and other procedures. If dried-egg powder is to compete with frozen-egg magma in the post-war era, it must perform as satisfactorily as the frozen-egg magma or must have decided advantages over the frozen-egg magma in some respects. This will require constant improvement of spray-dried eggs and variation in technics of use in products such as sponge cake.

One of the severest culinary tests of egg quality is its ability, when whipped into a foam, to aerate sponge cake. In the early stages of production of dried egg, the egg powder when reconstituted and whipped at room temperature seldom gave a foam sufficiently stable to produce acceptable sponge cakes. Then the English workers, Hawthorne and Bennion (1942) and Bennion, Hawthorne, and Bate-Smith (1942), reported that with addition to the egg powder of the sugar and water for reconstituting, and surrounding the container with a water bath at 50°C. (122°F.) during beating, a stable foam could be produced. The Western Regional Laboratory (1944) showed that little change took place in the egg powder when it was stored at a low temperature, —17.8°C.(0°F.). The same workers also found that powders which would not form stable foams at 45°C.(113°F.) might produce foams which would aerate sponge cakes, if the meringue infredients were preheated to 68°C.(154.4°F.) and beaten by surrounding the container with a water bath at 45°C.

This study shows the deterioration for stable-foam formation of a spray-dried whole-egg powder with accelerated aging. A higher meringue temperature than any previously reported is used to obtain a stable foam. In addition, the difference in initial quality of dried-egg powders is shown.

EXPERIMENTAL PROCEDURE

One powder, a spray-dried whole-egg (five per cent moisture), was obtained from a commercial source. The eggs from which the powder was

made had been prepared without preheating (pasteurizing). The powder was kept at —23.3°C.(—10°F.) until incubated for the accelerated storage tests. Three spray-dried whole-egg powders (less than two per cent moisture content) were obtained from three different plants operated by the same firm. The eggs used in the powders had been preheated before drying. The fifth whole-egg powder was prepared under vacuum from the frozen state ("vacuum-dried") in the Poultry Products Laboratory.

An estimate of the extent of the denaturation of the dried whole-egg powders was made. The method employed was essentially that described by Mirsky (1941) in which the proportion of total sulfhydryl groups exposed was the criterion of denaturation.

The fresh-egg-magma-sugar mix was prepared by bringing all meringue ingredients to room temperature, 25°C.(77°F.), placing them in a bowl of a Kitchen Aid electric mixer (Model G) and beating with the regulation whip. Beating was continued at high speed until a definite lightness of foam was obtained as determined by specific gravity (approximately 0.27), followed by beating at slow speed to equalize the air cells. The dry ingredients, sifted together three times, were folded into the meringue by standardized hand mixing. .

When egg powders were used, the egg-sugar-water mixture was slowly heated (10 minutes) to 68°C. The meringue ingredients were beaten by surrounding the container with a water bath at 45°C. Departures from this procedure will be noted in the appropriate sections.

Cakes were prepared, baked, and tested under standardized conditions. The following formula, developed in preliminary tests with the use of shell eggs, was used throughout:

Meringue:
    Egg magma...96.0 grams
    Sugar, granulated..50.0 grams
    Water, distilled.. 7.0 grams
    Lemon juice... 8.0 grams

Dry ingredients:
    Flour, cake...35.0 grams
    Sugar, granulated..30.0 grams
    Salt.. 0.5 grams

When egg powder was used it was substituted in the above formula, using one part by weight of egg powder (24 grams) to three parts of water.

Since baking powder is frequently used in sponge cakes by bakers, each cake treatment in the accelerated storage tests was repeated with addition of baking powder to the formula. When baking powder was used, one gram of sulfate-phosphate baking powder was added to the dry ingredients.

The cakes were baked in small rectangular pans with slightly sloping sides and 730 to 755 cubic centimeters capacity, into which 150 grams of batter were weighed. The cakes were baked in a preheated gas oven at 175°C.(346°F.) for 30 minutes. They were inverted and cooled one hour, after which baking losses were determined by difference in weight. Vol-

umes were measured by rape seed displacement before the cakes were removed from their tins. The cakes were wrapped in wax paper and stored overnight in a giant desiccator. They were rated subjectively by weighted scores. Five judges rated the cakes, each judge receiving a slice from the same position in each cake. The characteristics scored with possible score for each were as follows: crumb color 5, texture 35, tenderness 30, moistness 10, flavor 20, and total score (sum of all characteristics) 100. Objective tests of tenderness were made on the tensile strength apparatus.

Five replications were made for each treatment.

RESULTS

Accelerated Storage Tests: The dried-egg powder (five per cent moisture) used for these tests was taken from a large, hermetically sealed container and packed into small tin cans which were sealed. One-fourth of these cans was stored at —23.3°C. until used. The other cans were incubated at 37°C.(98.5°F.) for 7, 14, and 28 days. The changes in a dried-egg powder of five per cent moisture content in seven days at 37°C. are approximately equivalent to the changes occurring in the same powder during storage for a month at room temperature. Storage at 37°C. for 14 to 28 days may be equivalent to storage at room temperature for five to six months. In other words, deterioration of the egg powder is rapid at first, then slows down, so that the same ratio does not hold throughout all storage periods. In addition, the moisture content and the initial quality of the egg powder affect this ratio.

This egg powder was initially of poor quality and even before incubation would not form a foam with the Western Regional Laboratory procedure, i.e., by heating the ingredients of the meringue mix to 68°C. and surrounding the bowl in which the mix was beaten by a water bath at 45°C. A stable foam which produced an acceptable sponge cake was obtained by heating the meringue ingredients to 68°C. and keeping the water bath at 80°C.(176°F.) during the beating period.

A summary of the data on the spray-dried egg magma, foam, cakes, and palatability scores is given (Tables 1 and 2).

The intensity of the color of the egg powder increased with longer incubation of the powder; that incubated 14 and 28 days had a strong odor.

There was an increase in denaturation of the proteins, a reduction of foaming power, and a progressive decrease in pH of the egg magma with lengthening incubation of the egg powder. The extent of denaturation, as shown by the total sulfhydryl groups exposed, was 7, 38, 51, and 67 per cent for the egg-powder samples incubated 0, 7, 14, and 28 days, respectively. Foams of good volume were obtained from the egg powders incubated 0 and 7 days, but a foam was formed with difficulty from the powder incubated 14 days and the foam volume was not as great as that from the 0- and 7-day incubated powder. The egg powder incubated 28 days did not form a foam even with prolonged beating with the water bath at 80°C,

The volume and general appearance of the cakes from egg powder stored 0 and 7 days at 37°C. was good (Fig. 1). All had a good shape, a

TABLE 1

Average Value of Data for Egg Magma, Foams, and Cake Batters and Cakes From Foams From Fresh Shell Egg, Unaged and Aged Commercial, Spray-Dried, Whole Egg, and a "Vacuum-Dried" Egg Powder [1]

Kind of egg, days incubated, and baking powder addition	Meringue beating time, speed Number 3	Specific gravity		pH		Cake volume	Tensile strength
		Foam	Batter	Egg magma	Cake batter		
	sec.					c.c.	gm.
Spray-dried egg (5% moisture)							
0 days, no baking powder	300	.231	.361	8.36	4.89	591	40
0 days, baking powder added	.....	.....	.361	.....	5.49	624	33
7 days, no baking powder	325	.242	.350	8.12	4.85	564	34
7 days, baking powder added	.....	.....	.343	.....	5.41	591	28
14 days, no baking powder	504	.294	.437	7.67	4.81	479	44
14 days, baking powder added	.....	.....	.426	.....	5.48	525	40
28 days, no baking powder	1,200	.976	.992	7.26	4.79	185	....
28 days, baking powder added	(No foam)	.....	.941	.....	5.41	265	....
Shell egg	135	.262	.329	7.87	5.22	701	44
"Vacuum-dried" egg powder	236	.257	.353	8.72	5.02	653	40
Spray-dried egg (less than 2% moisture).							
Plant 1	204	.264	.360	8.25	5.03	610	47
Plant 2	132	.240	.319	8.36	5.04	681	40
Plant 3	193	.253	.346	8.36	5.04	629	44

[1] Meringues from the five per cent moisture content, spray-dried egg powder were doubled, then divided and baking powder added to one portion. Dried egg powders were reconstituted before taking pH of egg magma.
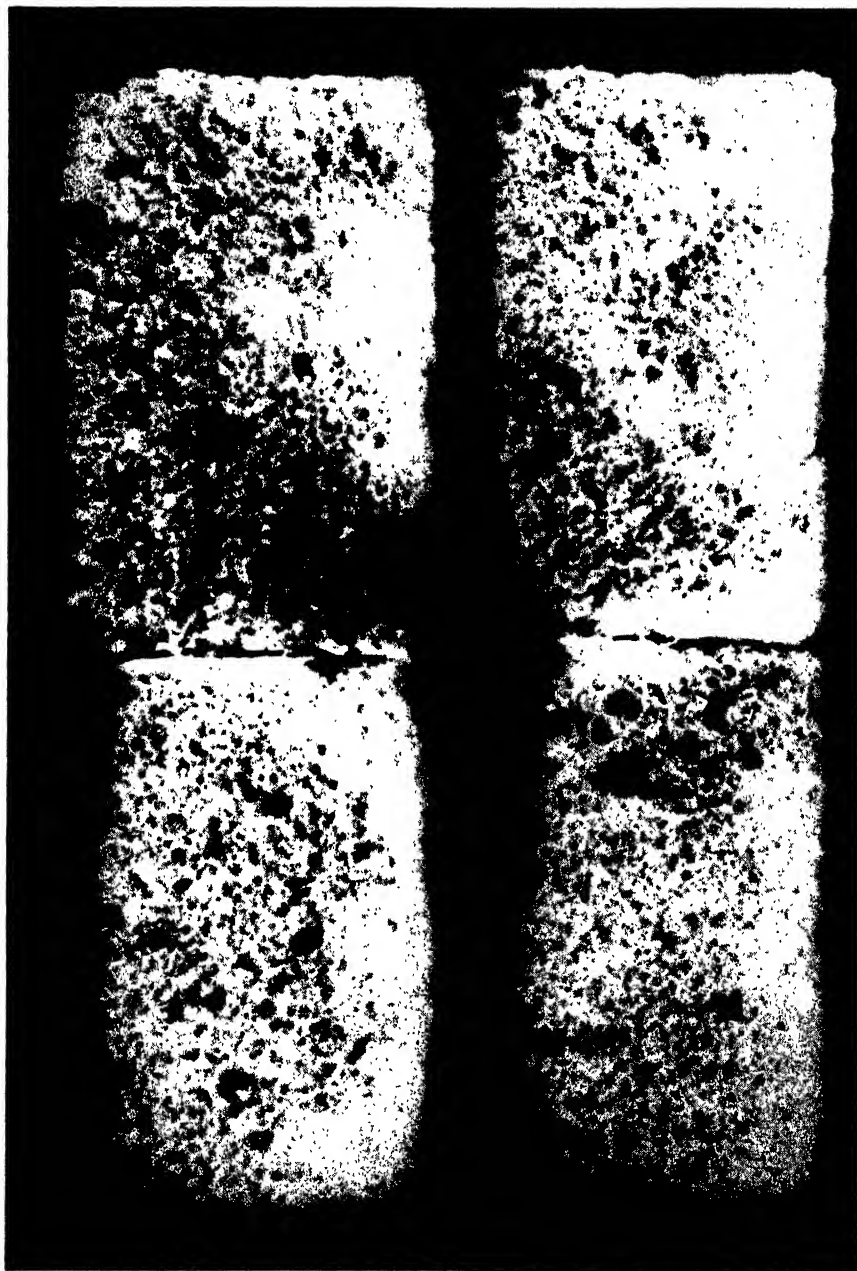
FIG. 1. Cakes made from commercial, spray-dried, whole-egg powder of five per cent moisture content.

Upper left: Not aged, baking powder added
Lower left: Not aged, control.
Upper right: Aged seven days, baking powder added.
Lower right: Aged seven days.

thin crust, and browned readily. With longer incubation, 14 and 28 days, the volume of the cakes made from the egg powder decreased. Cakes made with the powder incubated 14 days did not brown readily and tended to have thick, soggy crusts (Fig. 2). Cakes made with powder incubated 28 days were compact and soggy. Average total scores of the cakes decreased with lengthening incubation of the egg powder. Cakes made from the egg powder stored 14 days were considered unpalatable; those from the powder incubated 28 days, inedible.

TABLE 2

Average Cake Volume and Average Tenderness, Texture, Flavor, and Total Scores of Cakes Made From Liquid Shell-Egg Magma and Various Egg Powders

Egg and treatment	Days in-cubated at 37°C.	Scores			
		Tender-ness	Texture	Flavor	Total
Spray-dried egg powder, 5% moisture					
Without baking powder	0	24.3	26.6	14.2	78.4
With baking powder	0	23.8	24.8	13.9	75.2
Without baking powder	7	23.8	24.6	13.6	74.5
With baking powder	7	24.1	24.4	13.5	74.8
Without baking powder	14	20.5	17.6	9.8	58.8
With baking powder	14	21.6	20.3	10.9	64.4
Without baking powder	28	4.0	0.4	2.8	11.0
With baking powder	28	7.4	6.8	3.0	20.8
Liquid-egg magma from shell egg, control	....	25.9	29.1	16.7	84.9
Spray-dried egg powders, less than 2% moisture					
From Plant 1	....	24.5	26.4	14.3	78.5
From Plant 2	....	25.8	27.6	16.5	83.2
From Plant 3	....	24.8	27.0	15.0	80.5
"Vacuum-dried" egg powder	....	25.4	28.5	15.7	82.9

The addition of baking powder increased the pH of the cake batters, increased the volume and tenderness of the cakes, but had a variable effect on their palatability scores. The cakes with baking powder added scored lower in all characteristics than those in which it was omitted for the cakes made from the egg powder which was not incubated. They were similar for cakes made with or without baking powder from dried-egg powder incubated seven days. The cakes with baking powder scored higher than those from which it was omitted when dried egg which had been incubated 14 and 28 days was used. Jordan and Pettijohn (1946) recommend the addition of baking powder to sponge cake formula because of the increased tenderness and volume obtained in the cakes.

Powders From Different Plants: The three spray-dried egg powders were obtained from three different plants operated by the same company. All powders were refrigerated and used within a short time after they were received. The moisture content and an estimate of the extent of

FIG. 2. Cakes made from commercial, spray-dried, whole-egg powder of five per cent moisture content.

Upper left: Aged 14 days, baking powder added.
Lower left: Aged 14 days.
Upper right: Aged 28 days, baking powder added.
Lower right: Aged 28 days.

denaturation of the proteins were determined as soon as the sealed containers were opened. The values obtained follow:

Source of egg powder	Moisture (pct.)	Per cent of total sulfhydryl groups exposed
Spray-dried, Plant 1	1.6	13
Spray-dried, Plant 2	1.7	4
Spray-dried, Plant 3	0.7	22
"Vacuum-dried"	3.4	1

With greater denaturation of the egg proteins, a longer time was required to form a foam, the foam was not as stable as shown by reduced cake volume, and the total cake scores were lower. The meringue ingredients for the three spray-dried egg powders were combined as described under experimental procedure. However, the "vacuum-dried" egg-powder meringue ingredients were reconstituted at room temperature, then beaten with the water bath at 45°C. Thus the "vacuum-dried" egg was treated more as fresh-egg magma than the spray-dried egg. A summary of the data for the egg magma, the foams, and the cakes is given (Tables 1 and 2).

The differences in the initial quality of the dried-egg powders from different sources, as indicated by the extent of denaturation, were correlated with the total cake scores. The spray-dried egg from Plant 2, required a shorter beating time to form a foam, gave a more stable foam, and produced larger and more tender cakes than did the egg powders from Plants 1 and 3. The cakes also scored higher in texture and flavor. Analysis of variance of total scores showed that the three groups of cakes with the highest means—the shell-egg control (84.9), the spray-dried egg powder from Plant 2 (83.2), and the "vacuum-dried" egg (82.9)—differed significantly from the two groups of cakes with the lowest means—the spray-dried egg powder from Plant 3 (80.5) and from Plant 1 (78.5). There was no significant difference among the means within either of the two groups (Figs. 3 and 4).

One means of comparison of the strength or stability of the foams was to note the increase in specific gravity from foam to cake batter. Each foam was subjected to the same amount of mechanical manipulation in incorporating the flour. This increase in specific gravity was greater in batters with egg powders than when fresh liquid-egg magma was used, indicating a lowered ability of the dried-egg powder foams to withstand mechanical abuse.

In addition to improvement of the quality of the dried-egg powders, further improvement in the use of dried egg in sponge cake might be made by variation of the treatment of the meringue ingredients, variation in the proportions of ingredients in the formula, and substitution of cream of tartar for the lemon juice. Barmore (1934) reported that cream of tartar produces a more stable foam in angel cake than citric or acetic acids.

SUMMARY

The commercial, spray-dried egg powder of five per cent moisture content used in these tests (in common with most spray-dried powders) did
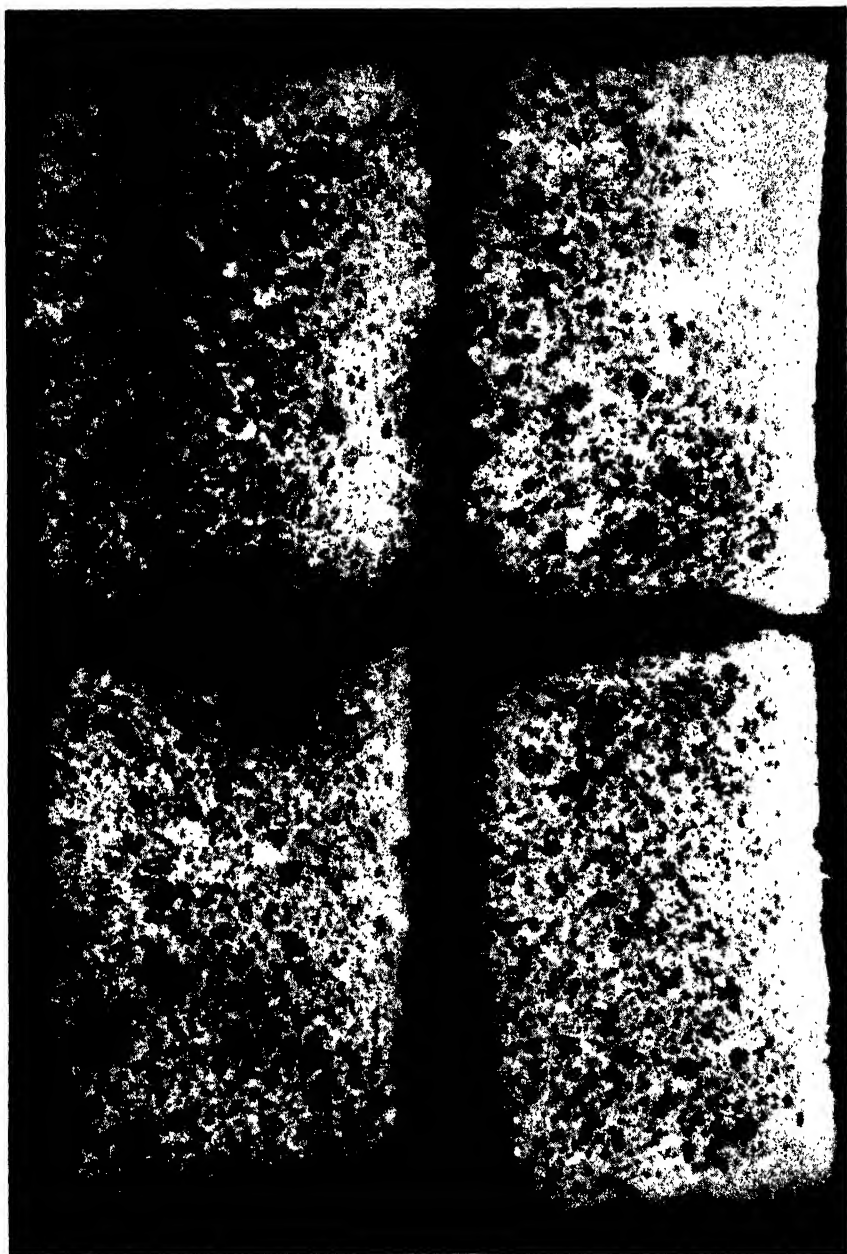
FIG. 3. Cakes made from dried whole egg. Three of the egg powders were from three different plants operated by the same firm and contained less than two per cent moisture.

Upper left: Egg powder from Plant 2.
Lower left: "Vacuum-dried" egg powder.
Upper right: Powder from Plant 3.
Lower right: Powder from Plant 1.

not form a foam when the powder was reconstituted and handled in the same manner as liquid shell-egg magma. A satisfactory foam and sponge cake was obtained by combining the meringue ingredients and slowly heating them to 68°C., then surrounding the electric mixer bowl with a water bath at 80°C., a water-bath temperature higher than any previously reported, during the whipping of the foam.
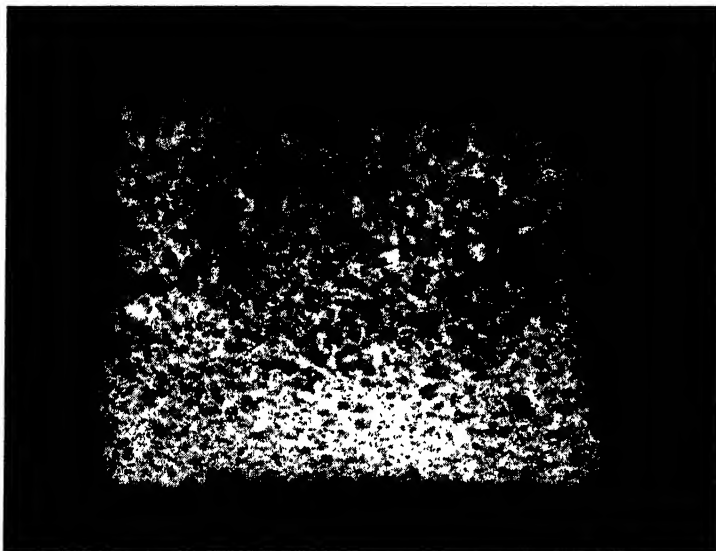
FIG. 4. Cake made from liquid shell-egg magma.

With accelerated aging at 37°C. for periods of 7, 14, and 28 days the commercial spray-dried egg powder showed the following progressive changes: an increase in denaturation of the egg protein (as shown by total sulfhydryl groups exposed), a decrease in pH of the egg magma, an increase in beating time with a reduction in foaming power, a reduction in cake volume, and a decrease in quality until the cakes made from the egg powder aged 28 days were considered inedible. Baking powder added to the cake formula with this egg powder increased the volume and tenderness of the cakes, increased flavor and texture scores of cakes from powders aged 14 and 28 days, but decreased the flavor and texture scores of cakes made from the unaged powder.

Freshly prepared, spray-dried, whole-egg powders of less than two per cent moisture content, which were obtained from three different plants operated by one firm, varied in initial quality as shown by extent of denaturation of the egg proteins, ease of foam production, cake volume, and cake quality. One of these egg powders and the powder dried from the frozen state under vacuum produced cakes comparable in quality to those made from fresh shell eggs. These three spray-dried egg powders produced acceptable cakes by heating the meringue ingredients to 68°C. and surrounding the mixer bowl during the whipping of the foam with

a water bath at 45°C. With the "vacuum-dried" powder the preliminary heating of the meringue ingredients to 68°C. was omitted.

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REDUCTONE INTERFERENCE IN ESTIMATION OF VITAMIN C

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Interference in the estimation of vitamin C caused by the presence of reductones and reductone-like substances that may be contained in certain types of processed foods has been reported by various workers. These substances, although structurally somewhat similar to vitamin C, possess no antiscorbutic activity and, as they reduce indophenol dye rapidly (in less than 15 seconds), it is important that they should not be mistaken for vitamin C.

Mapson (1943) devised a method whereby reductones can be differentiated from vitamin C by their reaction with formaldehyde. Under the conditions of his test, vitamin C condenses with formaldehyde whereas reductones do not. He presented evidence that reductones were produced when vegetables were exposed during dehydration to temperatures high enough to cause even slight scorching of the tissues, or when dehydrated products had been stored in air or nitrogen at temperatures exceeding 25°C.(77°F.). He particularly emphasized that when vegetables were dried under conditions known to avoid excessive damage to the tissues, interference from reductones was negligible. He found no substances of the reductone type in fresh vegetables and fruits, also that no reductones were produced in preserved or cooked foods as a general rule unless the temperature of the cooking was excessive, or unless the pH of the cooking water was above 8.5.

Wokes, Organ, and Jacoby (1943) reported interference in processed foods from reductones and other closely allied compounds, which they collectively termed "apparent vitamin C." Such interference was found particularly in foods high in carbohydrate and after prolonged storage under normal conditions. In their estimation of "apparent vitamin C," they employed a modified procedure of the formaldehyde method of Mapson (1943).

Robinson and Stotz (1945) presented a formaldehyde method for differentiating reductones from vitamin C that was much simpler and more adapted for control work in a food laboratory than that of Mapson. These authors noted large interference from reductones in food products that had been subjected to long heat treatments or extensive storage. They pointed out, however, that until the exact nature of reductones in different food products is known, their formaldehyde modification cannot be claimed to offer an exact differentiation between these substances and vitamin C. However, they believed it offered a closer approximation of the "true vitamin C" value in processed foods than measurements made hitherto by unmodified dye-reduction methods.

In the present study processed foods of various types were analyzed for vitamin C by both modified and unmodified dye-reduction procedures especially to determine extent of interference that may arise when reduc-

tones and reductone-like substances are present. A study of some fresh material has been included for control purposes.

EXPERIMENTAL PROCEDURE

Material: The test material comprised fresh vegetables and fruits and preserved, canned, concentrated, and dehydrated foods. Details regarding the nature of the samples used, including their treatment during storage, are indicated in the first column (Tables 1 and 2). All samples were laboratory samples unless marked "commercial." As honey represents almost pure dextrose and levulose, and as considerable controversy in the literature has been reported by Haydak, Palmer, Tanquary, and Vivino (1942) as to whether indophenol dye-reducing substances in honeys are to be identified with vitamin C, a study of four samples of honey was also included.

The history of the commercial samples of honey packed in Iowa and New York is not known. The Massachusetts samples had been centrifuged to remove the wax, heated to 49°C.(120°F.), strained through cheese-cloth, and immediately cooled to room temperature. These samples had been stored for approximately four months at room temperature, about 21°C.(70°F.), before they were received for analysis.

The fresh white potatoes were kept at 0°C.(32°F.) from harvest until May, when they were obtained from Aroostook County, Maine.

The fresh Florida orange juice was obtained from a Boston commercial supply house. The fresh cranberries were obtained from cranberry growers in Cape Cod, Massachusetts.

Of the commercial marmalades, one lot (dark) was prepared from Seville oranges and granulated sugar, whereas the other contained added pectin and acid, sliced oranges, and sugar. No further details are known.

Cranberry sauce, rose-hip jam, and orange marmalade were prepared in the laboratory, the formulae used being as follows:

CRANBERRY SAUCE
2 cups cranberries
1 cup water
1 cup sugar

Boil cranberries in water gently for eight minutes. Add sugar and simmer for five minutes. Pour into clean, hot jars and seal.

ROSE-HIP JAM
Cut rose hips in halves. Add water to cover and cook until tender. Rub through sieve. Heat one pound of strained fruit and one pound of sugar slowly to boiling, with constant stirring. Simmer for 30 minutes. Pour into clean, hot jars and seal.

ORANGE MARMALADE
Cut peel from six oranges into thin strips. Add three cups of boiling water and simmer for one hour. Add fruit, cut in small pieces, to the peel and six cups of sugar. Simmer for one hour. Pour into clean, hot jars and seal.

Methods of Analysis: Ascorbic acid (vitamin C) was determined by the indophenol-xylene extraction method of Robinson and Stotz (1945). The formaldehyde modification of this method, which corrects for the interfering action of reductones, was applied to all the materials analyzed. Ascorbic acid was also determined on uncolored extracts of fresh material

and on some of the uncolored, processed material by the indophenol dye-titration technique, the procedure being essentially that of Bessey and King (1933). The dye was standardized for the titration procedure by the method of Menaker and Guerrant (1938). The dye was delivered from a semi-micro, 10-ml. burette graduated in 0.05 ml. All titrations were completed within 10 seconds. A pink color appearing throughout the solution and persisting for at least five seconds was taken as the end point. Titrations were carried out in triplicate and the results averaged. A Fisher Daylight Lamp Titration Assembly was used. This gives a strong, uniform source of light that aids materially in detecting the correct end point. The extractant of ascorbic acid for both methods contained four per cent (by weight) of metaphosphoric acid. With potatoes, beets, and dehydrated carrots, however, a concentration of six per cent of metaphosphoric acid in the extractant was found more satisfactory for obtaining clear, non-foaming filtrates.

The principle of the indophenol-xylene extraction method of Robinson and Stotz (1945) depends upon the findings of Bukatsch (1939) that oxidized indophenol dye can be quantitatively extracted from an acid solution with xylene. The color of the extracted dye in xylene is stable provided there are no oxidizing substances in the xylene itself, as pointed out by Nelson and Somers (1945). The xylene used in this study was redistilled in an all-glass still and fractionated to remove impurities of oxidative nature, which would otherwise affect the reliability of the determination. Because of this stability of color, a series of extractions may be made and, when all are completed, the colors of the xylene extracts are measured in a photoelectric colorimeter. A Cenco photelometer, No. 12335, with Cenco No. 2 green filter transmitting maximally at 525 millimicrons, and Cenco fused absorption cells of one-cm. thickness and eight-ml. capacity were used for the photoelectric measurements. For carrying out the reactions, glass-stoppered reaction vessels with glass stopcocks, non-lubricant type, Pyrex (No. 7105-GR, Wilkens Anderson Company, Chicago, Illinois) were found convenient and also facilitated the separation of the xylene and aqueous phases. Ascorbic acid after the addition of buffer of pH 4.0 is unstable, as pointed out by Robinson and Stotz (1945) and Nelson and Somers (1945). Furthermore, at this pH the generally slower-acting, nonspecific, reducing substances may react fairly rapidly with the dye. Therefore, care was taken that, immediately upon buffering the ascorbic acid extract, the dye was added and mixed, followed quickly by xylene and vigorous shaking for 10 seconds, not more than 15 to 20 seconds elapsing during the entire procedure.

Many of the materials used in this study were highly colored, such as beets, cranberries, and strawberries. However, the indophenol-xylene extraction method is suitable for the determination of ascorbic acid in these foods because the pigments they contain are not soluble in xylene.

Robinson and Stotz (1945) have noted interference from reduced iron or tin, particularly in canned foods stored for long intervals. Therefore, the peroxide modification of their method that corrects for the dye-reducing action of these substances and for sulphite was applied in the analysis of all foods stored in cans as well as the formaldehyde modification.

In the dehydrated carrots xylene-soluble pigments were encountered. Correction for their presence was made by the hydroquinone-decoloration procedure of Robinson and Stotz (1945). Interference from xylene-soluble pigments is minimized by obtaining clear filtrates before proceeding with the indophenol-xylene extraction.

Total vitamin C, that is, ascorbic acid plus dehydroascorbic acid, was determined by hydrogen-sulphide reduction in some instances. This procedure, especially in the presence of reductones, is subject to great interference owing to the formation of hydroquinone types of compounds during the hydrogen-sulphide treatment. Robinson and Stotz (1945) have pointed out that these compounds condense with formaldehyde under the conditions of their test in the same manner as ascorbic acid and, therefore, no correction for the error caused by their presence can be made. King (1941) and Smythe and King (1942) called attention to large errors that might arise in the estimation of dehydroascorbic acid by hydrogen-sulphide reduction to form ascorbic acid. These errors were ascribable to the fact that many aldehydes, ketones, and quinones, when present in the ascorbic acid extracts, combined with hydrogen sulphide to form compounds that reacted like ascorbic acid under the conditions ordinarily used for titration or photoelectric estimations of vitamin C. In view of the findings of these authors and the large errors found in many of the cases where reductone interference was present, hydrogen-sulphide reduction to include the dehydro form was finally discontinued.

In the determination of total vitamin C by the indophenol-xylene extraction method, the hydrogen-peroxide modification was used in accordance with the suggestion of Rubin, Jahns, and Bauernfeind (1945) of the Hoffmann-La Roche Research Laboratories. This modification corrects for interference from residual traces of hydrogen sulphide that may remain in solution even though the extracts give negative tests with lead-acetate paper.

The moisture content was determined for all samples. The vacuum-oven method was used, and the samples were dried for six hours at 70°C. (158°F.) with about 30 inches of vacuum. The data on moisture content were used for calculation of the ascorbic acid on a basis of dry weight.

RESULTS AND DISCUSSION

Fresh Material: A summary of the vitamin C data for fresh vegetables and fruits, estimated by the indophenol-xylene extraction method with and without the formaldehyde modification and by the indophenol dye-titration technique (in cases where the material was not highly colored), is presented (Table 1). Interference from reductones and reductone-like substances was not found in any of the materials examined, as shown by the agreement of vitamin C values by modified and unmodified indophenol-xylene extraction procedures. Moreover, close agreement was shown between these values and those obtained by indophenol dye-titration technique.

No increases in vitamin C values, or negligible increases only, were noted in the fresh material after hydrogen-sulphide reduction, with the exception of freshly harvested beets. In the latter case, the vitamin C

TABLE 1

*Vitamin C Levels in Fresh Vegetables and Fruits by Different Methods **

Material	Moisture	Indophenol dye titration		Ascorbic acid			
				Indophenol-xylene extraction			
				No formaldehyde		With formaldehyde	
		Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.
	pct.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
Cabbage, Danish Ballhead, freshly harvested, Mass.	92.5	39.9	532	40.0	534	40.0	534
Beets, freshly harvested, Mass.	89.0	......	......	5.73	52.2	5.73	52.2
White potatoes, cold storage, 8 months, Maine	86.5	11.0	82.0	11.2	83.0	11.2	83.0
Sweet potato, market, October	68.3	31.2	98.4	30.6	96.5	30.6	96.5
Carrots, market, May	79.5	......	......	6.20	30.2	6.20	30.2
Green peas, market, May	76.8	22.1	95.3	22.5	97.0	22.5	97.0
Green peppers, market, May	92.5	97.8	1300	96.3	1280	96.3	1280
Whole tomatoes, market, May	94.6	13.4	248	14.3	265	14.3	265
Florida orange juice (Run 18) October	88.3	36.0	308	35.2	301	35.2	301
Florida orange juice (Run A) April	87.4	......	......	42.7	339	42.7	339
California orange juice and pulp (A), market, January	85.5	34.5	238	35.3	245	35.3	245
California orange rind (A)	73.6	60.3	228	60.6	229	60.6	229
California orange juice (B)	87.0	54.0	408	51.9	399	51.9	399
California lemon juice, market, May	92.1	38.9	492	38.3	486	38.3	486
Cranberries, Dec., Mass.	86.9	......	......	13.0	99.4	13.0	99.4
Strawberries, May, market	91.2	......	......	47.0	530	47.0	530
Rose hips, *Rosa nitida*, October, Mass.	79.6	885	4340	922	4500	922	4500

* The values reported in Tables 1 and 2 are accurate to the third significant figure.

value after hydrogen-sulphide reduction, estimated without the formalde-
hyde blank, was nearly twice the value obtained before hydrogen-sulphide
reduction. This increase in dye-reducing value, however, was found equal
to the value obtained for the formaldehyde blank (Fig. 1). Therefore, the
increased value was attributed to interference from a nonspecific dye-
reducing substance with a reductone-like structure formed in the beet
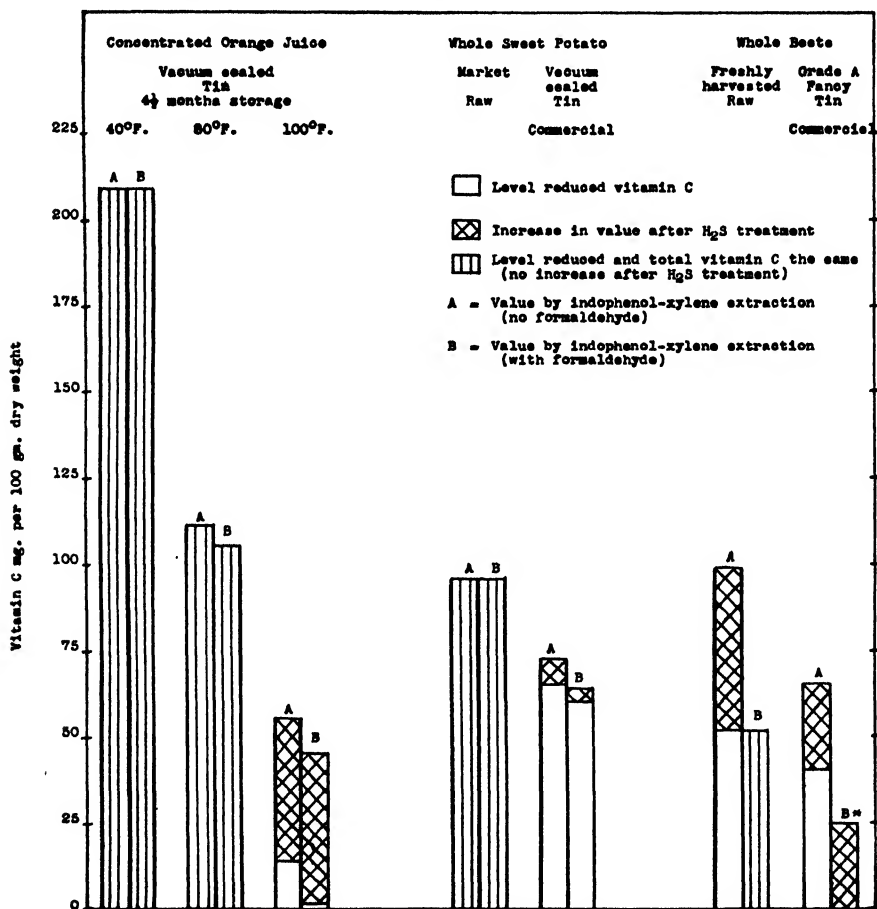
FIG. 1. Vitamin C levels by indophenol-xylene extraction, with and without formal-
dehyde, showing reduced vitamin C and increase in dye-reducing values obtained after
hydrogen-sulphide treatment.

    * Level reduced vitamin C=zero.

extract during the hydrogen-sulphide treatment. To consider this increased
value obtained by an unmodified method as dehydroascorbic acid would
appear to be erroneous.

    Processed Foods: A large number of processed foods was examined.
A summary of the vitamin C data obtained on the different groups of these
processed foods is presented (Table 2). The percentage of "true ascorbic
acid" for each sample has also been indicated. The reductone interference,

expressed as percentage, is the difference between 100 per cent and the percentage of the "true ascorbic acid."

In the group of preserved foods, all the commercial samples showed reductone interference with the exception of an orange marmalade that was noticeably dark in color when purchased. Whether the darkening was caused by excessive heat treatment during processing or by long storage was not known. The taste of this sample was good, although not as pleasing as the flavor of the freshly prepared product. Mapson (1943) and Robinson and Stotz (1945) found darkening to be associated with reductone interference. Therefore, it was suspected that this particular sample contained a reductone-like substance that condensed with formaldehyde in the same manner as ascorbic acid under the conditions of the method; hence no correction could be made for its presence. Errors in analyses from such sources have been pointed out by Mapson.

Our freshly preserved foods did not show any reductone interference. These foods were rose-hip jam, orange marmalade, and cranberry sauce containing, respectively, 337, 27.3, and 1.8 mg. of ascorbic acid per 100 grams. These samples showed extremely high, intermediate, and low values for the vitamin. When stored at $4°C.(40°F.)$, the rose-hip jam showed no reductone interference after six months, the orange marmalade showed reductone interference amounting to eight per cent of the dye-reducing substances after five months, and the cranberry sauce showed an interference of 100 per cent, that is, no "true vitamin C," after three months. Storage at $40°F.$, therefore, does not eliminate reductone development. It would appear, however, from these data that the extent of reductone development is inversely proportional, roughly, to the amount of vitamin C initially present in the samples. For example, the development of reductones was prevented in the rose-hip jam, for its high vitamin C content served to stabilize the product. There was only a slight interference from reductones in the orange marmalade, which contained initially a fair amount of vitamin C, but there was total interference in the cranberry sauce, which had a low initial vitamin C content. These results agree substantially with those of Lincoln and McCay (1945) who prepared a marmalade with an average initial vitamin C content of 28.6 mg. per 100 grams. Their product was studied for reductone development in storage by the formaldehyde method of Mapson (1943). After storage for eight months at $3°C.(38°F.)$, their samples did not show more than four to seven per cent reductone interference. However, their product was stabilized by cooking the peel in a solution of citric acid (0.2 per cent). These authors pointed out that in marmalade of high vitamin C content such as theirs, development of nonspecific substances of the reductone type was insignificant when storage was carried out at cool temperatures. However, with storage at elevated temperature the vitamin C content decreased and the reductones correspondingly increased.

In the case of the laboratory samples of cranberry sauce, the vitamin C content (no formaldehyde) apparently increased 81 per cent (wet-weight basis) during three and one-half months of storage. This illustrates the false impression of the stability of the vitamin that would be gained by use of an unmodified dye-reduction procedure. The taste of the cranberry

TABLE 2

Vitamin C Levels in Processed Foods by Different Methods

Material	Moisture	Indophenol dye titration		Ascorbic acid — Indophenol-xylene extraction method				
				No formaldehyde		With formaldehyde		"True ascorbic acid" Dry wt.
		Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	
	pct.	mg./100 gm.	mg./100 gm.	mg./100 gm.	mg./100 gm.	mg./100 gm.	mg./100 gm.	pct.
Preserved foods and honeys:								
Orange marmalade, initial, California fruit A	26.8	25.0	34.2	27.3	37.3	27.3	37.3	100
Orange marmalade, 3½ months, 40° F.	25.5	24.4	32.8	25.0	33.6	25.0	33.6	100
Orange marmalade, 5 months, 40° F.	25.6	24.8	33.3	25.3	34.0	23.4	31.5	92
Orange marmalade, commercial, light	31.7	11.0	16.1	12.1	17.8	10.8	15.8	89
Orange marmalade, commercial, dark	26.6	2.98	3.92	1.47	2.00	1.47	2.00	100
Cranberry sauce, initial, Cape Cod fruit	56.2	.....	.....	1.77	4.02	1.77	4.02	100
Cranberry sauce, 3½ months, 40° F.	54.9	.....	.....	3.19	7.08	0.0	0.0	0
Cranberry sauce, 5 months, 40° F.	55.7	.....	.....	2.86	6.46	0.0	0.0	0
Cranberry sauce, commercial, glass-packed	61.0	.....	.....	3.53	9.06	1.06	2.72	30
Rose-hip jam, initial, *Rosa nitida* fruit	38.0	336	543	337	544	337	544	100
Rose-hip jam, 3 months, 40° F.	37.2	339	540	339	540	339	540	100
Rose-hip jam, 6 months, 40° F.	35.3	318	492	310	480	310	480	100
Strawberry preserves, commercial	28.1	.....	.....	5.10	7.12	3.30	4.60	66
Strawberry preserves, commercial, 1 year, 80° F.	31.7	.....	.....	4.05	5.95	1.65	2.42	41
Honey, "mixed pollens," dark, Mass.	16.6	.....	.....	4.19	5.02	1.77	2.14	43
Honey, "linden," dark, Mass.	17.2	.....	.....	5.08	6.13	1.42	1.72	28
Honey, commercial, dark, New York	17.2	4.13	4.98	4.84	5.88	1.67	2.05	35
Honey, "white clover," light, Iowa	16.2	1.19	1.42	1.19	1.42	0.0	0.0	0
Canned foods:								
Whole sweet potato, commercial, vacuum-packed, Va.	68.1	22.3	70.0	21.1	66.2	19.2	60.2	91
Green peas, commercial, 1 year, 80° F.	83.3	10.9	65.2	10.6	63.7	8.47	50.8	80
Whole beets, commercial, 1 year, 80° F.	87.3	.....	.....	5.22	41.1	0.0	0.0	0
Whole tomatoes, commercial, 2 years, 80° F.	94.0	12.1	202	12.1	202	12.1	202	100
Pimientos, "fire-roasted," commercial, whole pods, Ga.								
Initial	91.0	78.1	867	.....	.....	.....	.....	.....
3 years, 80° to 95° F.	91.7	51.6	622	50.2	606	50.2	606	100
Pimientos, commercial, sliced pods, Ga, purchased May, 1946	92.8	65.5	910	57.5	800	57.5	800	100
Sauerkraut (A), commercial, purchased December, 1945								
Solids	92.0	17.6	220	17.1	214	6.10	76.3	36
Juice	94.5	18.6	338	17.5	319	17.5	319	100
Sauerkraut (B), commercial, purchased May, 1946.								
Solids	92.0	9.50	119	10.5	131	8.40	105	80
Juice	95.0	11.9	238	11.3	226	10.0	200	88

Food								
Sauerkraut (C), commercial, purchased May, 1946.								
Solids	92.2	3.58	46.0	4.17	51.8	1.59	20.4	39
Juice	95.2	1.79	37.3	1.59	33.2	0.0	0.0	0
Concentrated foods:								
Orange concentrate, commercial, vacuum-packed								
4½ months, 40°F	32.3	141	208	141	208	141	208	100
4½ months, 80°F	31.8	76.5	112	76.5	112	72.5	106	95
4½ months, 100°F	31.4	12.0	17.5	10.0	13.8	1.5	2.2	16
Orange concentrate (from Florida juice, run 18)								
Initial storage, screw-cap jars, 40°F	35.4	190	295	193	299	193	299	100
2 months' storage, screw-cap jars, 40°F	35.2	194	300	189	292	189	292	100
3 months' storage, screw-cap jars, 40°F	35.2	178	275	192	297	175	270	91
Tomato paste, commercial, tin, 1 year, 80°F.								
After 2 weeks, screw-cap jars, 40°F	75.0			6.83	27.3	3.87	15.5	57
After 2 weeks, screw-cap jars, 100°F	73.8			7.03	26.9	0.0	0.0	0
Dehydrated foods:								
Beets, julienne, commercial, N. J., 3½ years, 80° to 95°F	4.77			37.3	39.2	0.0	0.0	0
Cabbage shreds, commercial, vacuum-packed, California, 2½ years, 80° to 95°F	4.67	54.0	56.8	66.6	70.0	21.6	22.7	32
Carrots, diced, commercial, vacuum-packed, California, 3 years, 80° to 95°F, (tunnel-dried)	5.63			32.1	34.0	18.5	19.6	58
White potato, julienne, commercial, V-board packed, Maine, 1½ years, 80° to 95°F	6.36	23.3	24.9	23.5	25.1	23.5	25.1	100
White potato slices, Maine potatoes								
Tunnel dried, light	6.57	23.4	25.1	23.0	24.6	23.0	24.6	100
Tunnel dried, some dark areas	6.57	16.5	17.7	16.2	17.4	16.2	17.4	100
Orange-juice powder, California Valencia fruit								
Commercial screw-cap jars, initial	1.63	334	340	330	336	330	336	100
Commercial screw-cap jars, 5 months, 80°F	1.61	328	333	318	323	318	323	100
Commercial screw-cap jars, 8 months, 80°F	1.61	355	362	373	380	318	323	85
Orange-juice powder, Florida fruit, commercial								
Dried by vacuum diffusion, initial	1.94			255	260	255	260	100
Dried by vacuum diffusion, 3 months, 80°F. (caked)	2.43	255	261	255	261	255	261	100
Stored desiccator, CaCl₂, 7 months	0.47			280	282	280	282	100
Orange-juice crystals, freeze-dried, commercial								
Initial storage	0.79	375	378	343	344	343	344	
Stored desiccator, CaCl₂, 2 years	0.39							100
Lemon powder, freeze-dried, compressed								
Initial storage	1.63	360	366	102	104			
Stored screw-cap jars, 3 years, 80°F	2.22	91.3	95.4			59.3	60.3	58
Orange-juice crystals (Run CD) vacuum-belt dried	1.28			327	335	327	335	100
Orange-juice crystals (Run A) slightly scorched, vacuum-belt dried	2.28			320	328	284	291	89

sauces was excellent, their fresh-fruit flavor being retained in spite of total reductone interference.

In all the samples of honey there was great interference from reductones, as would be expected in a material of such high carbohydrate content. One sample contained no "true vitamin C," whereas three samples showed an average of 65 per cent reductone interference. Levels for vitamin C were low and ranged from 0 to 1.8 mg. per 100 grams (wet weight) of the reduced form of the vitamin. Haydak, Palmer, Tanquary, and Vivino (1942) made an extensive study of the vitamin content of various types of honey obtained from Minnesota, territorial United States, and foreign countries. They determined the vitamin C content by the method of Bessey (1938). Values for vitamin C obtained by these authors ranged from 0.5 to 6.5 mg. per 100 grams and averaged 2.2 mg. per 100 grams. The pronounced variability in the vitamin C content, they believed, was ascribable to differences in the floral source and the number of pollen grains in the product. In no case did they obtain values for vitamin C in foreign honeys as high as has been reported in the literature. In view of the findings of these authors and our own findings, it would appear that reductone interference has been largely responsible for the discrepancies in the vitamin C values reported for different honeys.

Analyses of total vitamin C were undertaken on all preserved foods and the honeys. Large increases in dye-reducing values after hydrogen-sulphide treatment were found in the honey samples, the cranberry sauces, the strawberry preserves, and the commercial, dark orange marmalade (Fig 2). With respect to the freshly prepared cranberry sauce, the formaldehyde blank eliminated the increase in dye-reducing values after hydrogen-sulphide treatment. This was also the case in the fresh-beet samples (Fig. 1). The increases in dye-reducing values after hydrogen-sulphide treatment in the other samples were not eliminated, or only partially, by the formaldehyde blank. However, these increases were attributed not to dehydroascorbic acid but to hydroquinol types of compounds that condense with formaldehyde in the same manner as ascorbic acid and hence lead to "apparent dehydroascorbic acid" values. The freshly prepared orange marmalade, with which no reductone interference was noted, gave only a negligible increase in dye-reducing value after the hydrogen-sulphide treatment. The strawberry preserves in this group (commercial samples only) were low in vitamin C and showed large interference from reductones. The sample stored for one year at 26.7°C. (80°F.) contained half as much vitamin C as the sample analyzed directly after purchase. Furthermore, the reductone interference was 25 per cent greater in the one-year sample than in the sample analyzed directly after purchase. In respect to taste the stored sample was inferior to the sample representing no storage and also had a slight off-color.

A comparison of the dye-titration values with the "true vitamin" values in the group of preserved foods and honeys shows that where reductone interference was present the former method gave values far too high. However, where there was no interference from reductones, as in freshly prepared orange marmalade and the rose-hip jams, close agreement between the "true vitamin C" and dye-titration values was obtained.
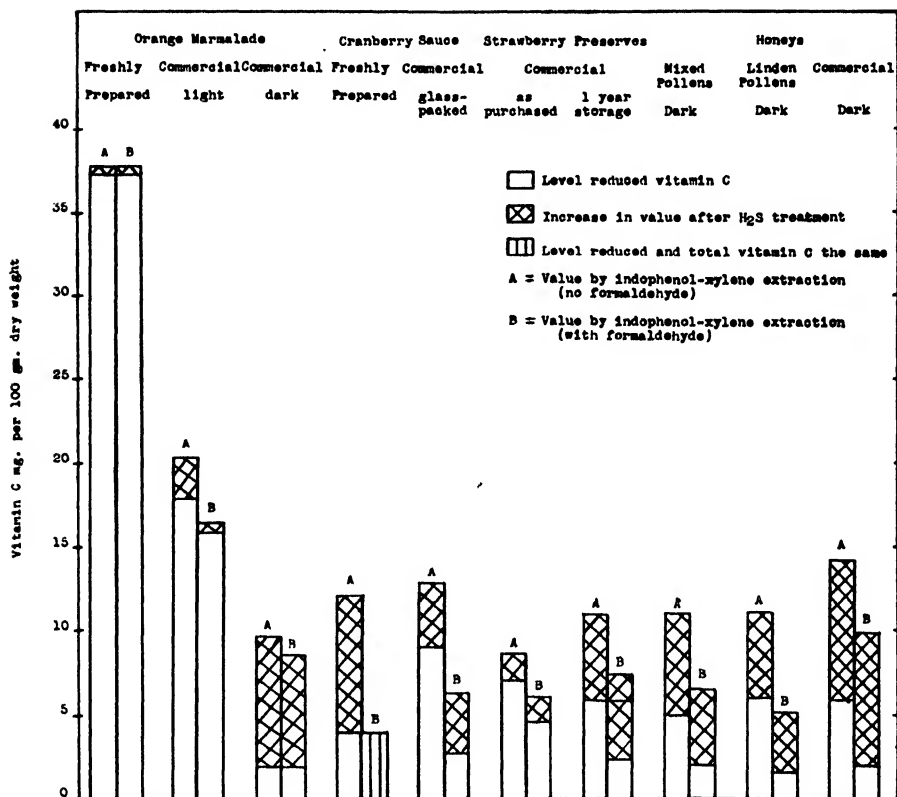
Fig. 2. Vitamin C levels by indophenol-xylene extraction, with and without formaldehyde, showing reduced vitamin C and increase in dye-reducing values obtained after hydrogen-sulphide treatment.

In the commercially canned vegetables reductone interference was found in sweet potato (nine per cent), green peas (20 per cent), and beets (100 per cent), whereas none was found in tomatoes and pimientos. The sweet potato was analyzed directly after purchase and was an excellent product with good retention of flavor. The peas and beets had been stored for one year at 80°F. The flavor of the beets was good and that of the peas fairly good. The tomatoes and pimientos had been stored for two and three years at high temperatures, but in spite of their rigid treatment the pimientos, which were a "fire roasted" product, had retained their original color and flavor.

A comparison of the vitamin C values of these canned products emphasizes, as in the group of preserved foods, that the higher the vitamin C content, the less was the interference from reductones. For example, with a low source of vitamin C, such as beets, total interference was found, which was also the case with the cranberry-sauce samples, whereas with pimientos, which constitute a rich source of vitamin C, no reductone interference was found even after prolonged storage at high temperature. During this long storage interval, however, the pimientos lost some vitamin C but still retained 72 per cent of the original content. Positive values for

reduced iron and tin were also obtained on the pimientos stored for three years. The value for vitamin C obtained by the indophenol-xylene extraction procedure without the formaldehyde modification and without the peroxide modification was the same as that obtained by the dye-titration technique. Therefore, in the latter method the titer included all the vitamin C plus all the reductones and the reduced iron and tin.

Hydrogen-sulphide reduction for the purpose of including the dehydroascorbic acid gave large increases over the reduced form with beets and moderate increases with sweet potatoes (Fig. 1). With pimientos and tomatoes, however, no increases in dye-reduction values occurred after the hydrogen-sulphide treatment.

Included in the group of canned products were three different commercial samples of sauerkraut indicated as A, B, and C (Table 2). Both solids and juice were examined. All showed reductone interference except the juice of Sample A. Considerable variation in the "true vitamin C" content was noted, and much higher values were obtained by the unmodified method. The vitamin C content of the juice was higher than that of the solids for Samples A and B, but for Sample C a zero value was obtained in the juice. Sample A showed 64 per cent reductone interference in the solids and none in the juice; Sample B, 20 per cent in the solids and 12 per cent in the juice; and Sample C, 61 per cent in the solids and 100 per cent in the juice. The history of the samples was not known. Sample A was purchased in December and Samples B and C in May, and analyses were made immediately after purchase. All were excellent sauerkrauts, Samples A and B having exceptionally fine flavor.

Pederson, Mack, and Athawes (1939) found considerable variation in the vitamin C content of canned sauerkrauts, the majority of the samples showing slightly higher values for the juice than for the solids. Their range for vitamin C in canned sauerkraut was from nine to 31 mg. per 100 grams of juice or solids. Vitamin C was determined by the method of Bessey and King (1933) as modified by Mack and Tressler (1937). They found canned sauerkraut contained less vitamin C than the raw product but noted little decrease during storage. The loss in vitamin C, they believed, took place primarily in the mixing and preheating vat before the sauerkraut was put into the cans. In view of their findings, the explanation for the wide variation in reductone interference in our samples would appear to be ascribable to differences in processing treatments before canning rather than to possible differences in storage conditions after canning.

In the group of concentrated foods, reductone interference was noted in certain of the stored samples (Table 2). In the samples of commercial orange concentrate held for four and one-half months in vacuum-sealed cans, no interference was found in the sample stored at 4°C.(40°F.), five per cent interference was found in the sample held at 27°C.(80°F.), and 84 per cent in that held at 38°C.(100°F.). Corresponding values for "true vitamin.C" were 141, 72.5, and 1.5 mg. per 100 grams (wet weight). In other words, with increasing storage temperature accompanied by falling vitamin C values, interference from reductones correspondingly in-

creased. These samples also darkened progressively, for the sample at 80°F. had changed slightly in color and that at 100°F. was very dark and had a suggestion of fermented odor. Therefore, the increase in reductone interference also corresponded to the progressive change in color from light to dark. Hence, although vacuum sealing may have retarded reductone development, it did not eliminate it. In the case of the Florida orange concentrate stored in screw-cap jars at 40°F., no interference from reductones was encountered until the third month. Then the interference was slight (nine per cent).

Treatment with hydrogen sulphide gave large increases in dye-reducing values in the commercial orange concentrate held at 100°F., but no increases in those samples held at 40 and 80°F. (Fig. 1). No correction could be made for the increase in value obtained by the unmodified method in the sample held at high temperature. The increase, however, was considered to be only "apparent dehydroascorbic acid" and is attributed to interference from hydroquinol types of compounds, which have been previously described.

Hydrogen-sulphide treatment of the Florida orange concentrate stored at 40°F. gave an increase of 12 per cent in the dye-reducing values after two months of storage and of three per cent after three months (Fig. 3). In both cases almost complete correction for these increases was made by the formaldehyde blank. Similar findings were noted for fresh beets (Fig. 1) and freshly prepared cranberry sauce (Fig. 2).

Included in the group of concentrated foods was a commercial tomato paste that had been stored for one year at 80°F. To observe the effect of storage at high temperature, the tin in which this product was held was opened and half the sample was placed at 40°F. and the other half at 100°F. After two weeks, slight browning was observed in the latter sample. Reductone interference amounted to 43 per cent in the sample held at 40°F. and to 100 per cent in that held at the higher temperature, that is, the sample in which slight but definite browning had occurred. Without the formaldehyde blank correction the values for the samples held at 40 and 100°F. were essentially the same, which illustrates the apparent stability that would be reported in the absence of a modified procedure to correct for reductone interference.

In the group of commercially dehydrated foods the vegetables all showed reductone interference with the exception of potato. This interference amounted to 42 per cent in the carrots, 68 per cent in the cabbage, and 100 per cent in the beets. The length of storage for these samples represented from one and one-half to three and one-half years. The temperature range over these periods was 80 to 95°F. In spite of the long storage at elevated temperatures, however, the carrots and potatoes (julienne style) were still in good condition, their color and general appearance resembling freshly dehydrated material, and the cooked products were highly edible. The low moisture content and the vacuum packing undoubtedly were important factors in prolonging the storage life and the edibility of the carrots. The dehydrated beets had retained their natural color and were still edible, although the flavor of the cooked product could
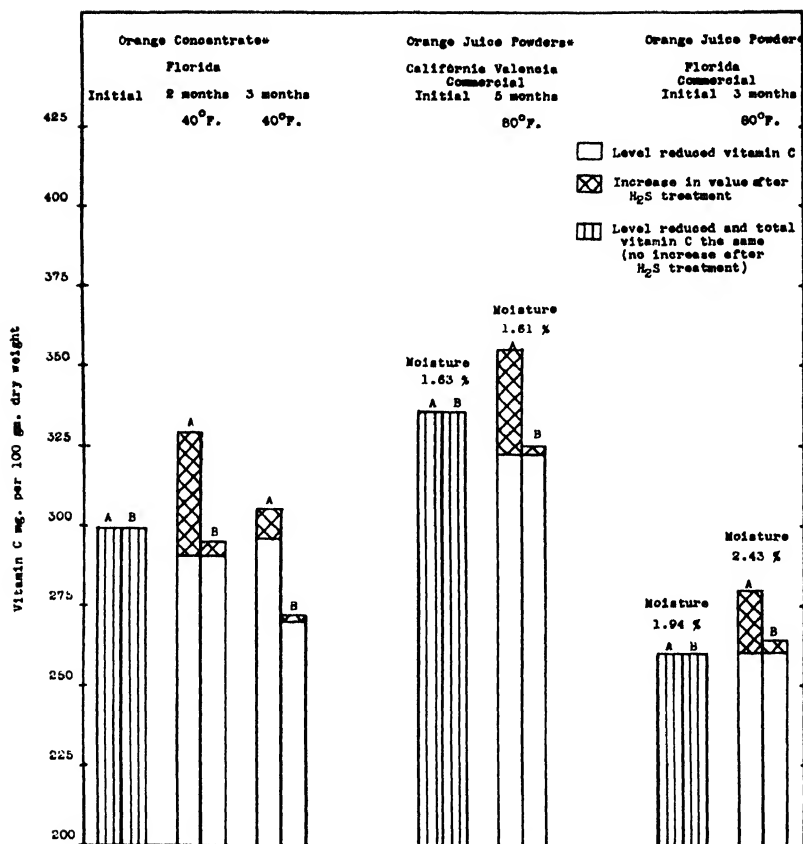
Fig. 3. Vitamin C levels by indophenol-xylene extraction, with and without formaldehyde, showing reduced vitamin C and increase in dye-reducing values obtained after hydrogen-sulphide treatment.

A=value by indophenol-xylene extraction (no formaldehyde).

B=value by indophenol-xylene extraction (with formaldehyde).

* Storage in screw-cap jars.

not be identified with that of freshly cooked beets. The cabbage shreds had a stale or hay odor, and some of the shreds were light brown in color. The sample had been treated with sulphite; however, when the can was opened, tests for the presence of sulphite were negative. This sample was not edible.

Two samples of white potatoes from Maine, which had been held in cold storage during the winter, were tunnel dried and then examined. The vitamin C level for the first of these two samples (light colored) was essentially the same as that obtained for the commercially dehydrated Maine potatoes (julienne style). One lot of the tunnel-dried potatoes showed some darkened areas on the slices. Analyses for vitamin C, however, showed no measurable reductone interference, although a loss of vitamin C amounting to 29 per cent had occurred.

In the dehydrated citrus-fruit products, reductone interference amounting to 11 per cent was found in a vacuum belt-dried orange-juice sample that had been slightly scorched in the drying process. Reductone interference was also found in samples after long storage in screw-cap jars at 80°F., this interference amounting to 15 per cent in California orange-juice powders after eight months and 42 per cent in freeze-dried compressed lemon powder after three years. In the latter sample a slight off-color was noted, and the moisture content was slightly greater than the initial value. In the California sample, however, which represented a much shorter storage period, no change in color and no increase in moisture content occurred. In samples of orange-juice powders stored in desiccators over calcium chloride, no interference from reductones was found in the Florida orange-juice powder stored for seven months or in the freeze-dried orange-juice crystals stored for two years. Both samples had moisture contents of less than 0.5 per cent, and the rehydrated product of the latter sample had a flavor closely resembling that of fresh orange juice. The maintenance of a low moisture content or the taking up of the "bound water" in the citrus-fruit powders by the desiccant during storage was undoubtedly an important factor in the prevention of reductone interference, the retention of fresh-fruit flavors, and the retention of vitamin C.

Treatment with hydrogen sulphide gave increases in dye-reducing values in stored citrus-fruit powders, namely, 10 per cent for California Valencia orange-juice powder and seven per cent for Florida orange-juice powder (Fig. 3). In both cases, almost complete correction for these increases was obtained in the formaldehyde blank. These findings are similar to those for Florida orange concentrates in storage (Fig. 3).

SUMMARY AND CONCLUSIONS

A study of fresh and processed foods was undertaken to determine the extent of interference that may arise in the estimation of vitamin C when reductones and reductone-like substances are present. The formaldehyde modification of the indophenol-xylene extraction procedure of Robinson and Stotz (1945), which corrects for interfering action from reductones, was used for the vitamin C estimations, and the results were compared with the unmodified, indophenol-xylene extraction technique and (in cases of uncolored material) with the dye-titration technique.

No reductone interference was encountered in fresh vegetables and fruits, and the values obtained for vitamin C by the three techniques were in close agreement.

Reductone interference was encountered in all the processed foods studied, including preserved foods, honeys, and canned, concentrated, and dehydrated foods.

A comparison of the vitamin C values obtained for processed foods by the formaldehyde procedure and the dye-titration technique indicated that where reductone interference was present the dye-titration technique gave values far too high, whereas in the absence of reductone interference close agreement between the two methods was obtained.

With practically all the commercial preparations of preserved foods there was reductone interference, ranging from 11 to 70 per cent.

With the freshly prepared, preserved foods there was no reductone interference. After storage at 40°F., however, reductone interference was noted, the extent of the interference being roughly inversely proportional to the initial vitamin C content. In the samples with high vitamin C content, there was no reductone interference after six months; in those with a fair amount of vitamin C initially present the interference was about eight per cent after five months; and in those with low initial vitamin C content 100 per cent (no "true vitamin C") after three months.

All the honey samples showed large reductone interference. One sample contained no "true vitamin C," and an average reductone interference of 65 per cent was noted in the other samples.

With the exception of tomatoes and pimientos all the canned products (commercial only) showed reductone interference, ranging from nine per cent in sweet potatoes (storage conditions unknown) to 100 per cent in whole beets stored at 80°F. for one year.

Reductone interference in concentrated foods seems to increase progressively with increase in the temperature of storage. The increase in reductone interference also corresponded to the progressive changes in color of the samples from light to dark.

In all the commercially dehydrated vegetables except potatoes there was reductone interference, ranging from 42 per cent in carrots to 100 per cent in beets.

Reductone interference was noted in dehydrated citrus-fruit products, amounting to 11 per cent in orange juice that had been slightly scorched in the drying process and 15 to 42 per cent in citrus-fruit powders stored for eight months and three years, respectively, in screw-cap jars.

No reductone interference was found in freeze-dried orange-juice crystals that had been stored in a desiccator over calcium chloride for two years. The fresh-fruit flavor and the vitamin C content of the original product had been retained.

Hydrogen-sulphide reduction for the purpose of including dehydro-ascorbic acid gave no increases or negligible increases only in dye-reducing values for fresh vegetables and fruits, with the exception of freshly harvested beets. The dye-reducing value for the beets with hydrogen-sulphide treatment was nearly twice that obtained before hydrogen-sulphide reduction. This increase in the dye-reducing value, however, was eliminated by the formaldehyde blank and was attributed to interference from a non-specific dye-reducing substance with a reductone-like structure formed in the beet extract during the hydrogen-sulphide treatment. To consider this increased value obtained by the unmodified method as dehydroascorbic acid would appear to be erroneous.

Large increases in dye-reducing values after hydrogen-sulphide treatment were found in the majority of the preserved foods and in all the honey samples. With the freshly prepared cranberry sauce, the increase in dye-reducing values was eliminated by the formaldehyde blank. The increases in dye-reducing values in the other samples, however, were not eliminated, or only partially, by the formaldehyde blank.

Hydrogen-sulphide treatment gave moderate increases in dye-reducing values for canned sweet potatoes, large increases for canned beets, and no increases for canned tomatoes and pimientos. Increases in dye-reducing values were also noted with orange concentrates and dehydrated orange-juice powders after storage. With certain of the orange concentrates and with the orange-juice powders, these increases were almost entirely eliminated by the formaldehyde blank.

These increases, for which there was no correction by the formaldehyde blank, are attributed not to dehydroascorbic acid but to hydroquinol types of compounds that condense with formaldehyde in the same manner as ascorbic acid and hence lead to "apparent dehydroascorbic acid" values.

"Apparent" stability or increases in vitamin C were noted in many processed foods in storage, when the vitamin C was estimated by unmodified dye-reduction procedures, whereas actually no "true vitamin C" was present. This illustrates the serious errors that may be introduced in the absence of a method that corrects for the nonspecific action of reductones.

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COMPARATIVE STUDIES ON GROWTH AND BIOCHEMICAL FEATURES OF MICROORGANISMS GROWN IN COW'S AND SOYBEAN MILK

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Morse and Piper (1923) reported that soybean or vegetable milk was originated by the Chinese philosopher, Whai Nain Tze, long before the Christian era and is known to the Chinese under the name of Fu Chiang, or bean curd sauce. This vegetable milk is used not only in the fresh state by the Chinese but the Japanese condense it by a process similar to the concentration of cow's milk. Vegetable milk is extensively used throughout China for infant feeding as well as a food for adults.

The introduction of soybean milk to the American people has occurred within the past few years. Attempts are being made to manufacture this milk in the form of a powder; it has been used with good results in bread and cakes, in creaming vegetables, in custards, in chocolate or cocoa, and in several other food products as a substitute for cow's milk. The high nutritive value of soybean milk and its many potential uses indicate that this product will continue to rise in importance in the human diet.

The following comparative figures show the composition of cow's and a typical soybean milk as published by the Battle Creek Food Company (1937):

Composition	Soybean milk (per cent)	Cow's milk (per cent)
Protein	3.60	3.30
Fat	1.52	4.00
Carbohydrates	5.00	5.00
Calcium	0.025	0.114
Iron	0.0010	0.0002
Ash	0.79	0.70
Calories per ounce	13.7	19.7

It may be seen from the figures given that the composition of soybean milk closely resembles that of cow's milk. The iron content of soybean milk is about five times that of cow's milk, while the calcium content is considerably lower, although it compares with the calcium content of human milk.

From a bacteriological viewpoint, however, the sale and development of soybean milk on a commercial scale present new technical problems, the importance of which is as yet undetermined. This investigation was undertaken in an attempt to determine the comparability of various biochemical reactions produced in cow's and soybean milk when inoculated with *Lacto-*

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bacillus bulgaricus, Lactobacillus acidophilus, Streptococcus liquefaciens, Streptococcus lactis, Aerobacter aerogenes, and *Escherichia coli.*

The test organisms used in this investigation were obtained from the stock culture collection maintained in the Department of Bacteriology, The Ohio State University. The cultures were carried in litmus milk, transferred once a week, and incubated at 37°C.(98.6°F.) until coagulation occurred. The cultures were removed immediately after coagulation and held at 5°C.(41°F.). In no case were inoculations made directly from the stock cultures, but instead, transfers were made from new cultures which were prepared from the stock cultures. The stock cultures were examined microscopically every two weeks to determine their purity.

Sterile aqueous solution of litmus was added to fresh skim milk until a robin's egg blue color was obtained, and then the milk was sterilized at 100°C.(212°F.) for 20 minutes on three consecutive days. The soybean milk used was obtained from Dr. Harry Miller, Director of the International Nutrition Laboratory, Mt. Vernon, Ohio. The method used for the preparation of soybean litmus milk was similar to the method employed in the preparation of cow's litmus milk.

Approximately 10 c.c. of the cow's and soybean litmus milk were inoculated with 0.1 c.c. of a two-day old culture of the test organisms, then incubated at 37°C. for 24, 48, 72, and 96 hours. All samples of milk were inoculated with the test organisms and incubated at 37°C. for the respective incubation periods, except *S. lactis,* which was incubated at 21°C. (69.8°F.). The biochemical reactions were noted at the end of each incubation period; results are presented (Table 1).

Micrometric measurements of the test organisms grown in the two kinds of milk were studied with the aid of a Bausch and Lomb stage and ocular micrometer. The direct microscopic staining procedure was used as suggested in "Standard Methods for the Examination of Dairy Products" (1939).

The hydrogen-ion concentration was determined on 10-c.c. samples of each type of milk inoculated with the test organisms and incubated at 21 or 37°C. for 0, 18, 48, 72, 96, and 120 hours. A Coleman 3C glass electrode potentiometer was used. The data are presented (Table 2).

Growth of *L. bulgaricus* in soybean litmus milk showed that coagulation occurred more rapidly, whereas in litmus milk acid production and litmus reduction were more pronounced (Table 1-a). It was observed upon growth of *L. acidophilus* in soybean litmus milk that coagulation did not occur until after an incubation period of 48 hours, while in litmus milk coagulation was evident after 24 hours. Likewise, reduction of litmus was incomplete after 96 hours in the soybean litmus milk, while in litmus milk reduction occurred after 24 hours, and peptonization did not occur in either sample (Table 1-b). *S. liquefaciens* produced marked peptonization of litmus milk while in soybean milk this reaction was only slightly evident. Acid production, coagulation, and reduction of litmus occurred to the same

TABLE 1

Biochemical Reactions Produced in Cow's and Soybean Litmus Milk When Inoculated With L. bulgaricus, L. acidophilus, S. liquefaciens, S. lactis, A. aerogenes, and E. coli [1]

	a. Cow's milk inoculated with *L. bulgaricus* and held at 37°C.				a. Soybean milk inoculated with *L. bulgaricus* and held at 37°C.			
	24 hr.	48 hr.	72 hr.	96 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Coagulation	—[2]	—	x	x	—	x	x	x
Reduction of litmus	*	x	x	x	—	*	x	x
Acid production	*	x	x	x	*	*	x	x
Peptonization	—	—	—	—	—	—	—	—
Controls	—	—	—	—	—	—	—	—
	b. Cow's milk inoculated with *L. acidophilus* and held at 37°C.				b. Soybean milk inoculated with *L. acidophilus* and held at 37°C.			
Coagulation	—	x	x	x	*	*	x	x
Reduction of litmus	*	x	x	x	*	*	*	*
Acid production	*	x	x	x	x	x	x	x
Peptonization	—	—	—	—	—	—	—	—
Controls	—	—	—	—	—	—	—	—
	c. Cow's milk inoculated with *S. liquefaciens* and held at 37°C.				c. Soybean milk inoculated with *S. liquefaciens* and held at 37°C.			
Coagulation	x	x	x	x	x	x	x	x
Reduction of litmus	x	x	x	x	x	x	x	x
Acid production	x	x	x	x	x	x	x	x
Peptonization	x	x	x	x	—	—	—	*
Controls	—	—	—	—	—	—	—	—
	d. Cow's milk inoculated with *S. lactis* and held at 21°C.				d. Soybean milk inoculated with *S. lactis* and held at 21°C.			
Coagulation	x	x	x	x	*	x	x	x
Reduction of litmus	x	x	x	x	*	x	x	x
Acid production	x	x	x	x	*	x	x	x
Peptonization	—	—	—	—	—	—	—	—
Controls	—	—	—	—	—	—	—	—
	e. Cow's milk inoculated with *A. aerogenes* and held at 37°C.				e. Soybean milk inoculated with *A. aerogenes* and held at 37°C.			
Coagulation	—	—	*	*	x	x	x	x
Reduction of litmus	*	x	x	x	—	—	—	*
Acid production	*	*	*	x	x	x	x	x
Peptonization	—	—	—	*	x	x	x	x
Controls	—	—	—	—	—	—	—	—
	f. Cow's milk inoculated with *E. coli* and held at 37°C.				f. Soybean milk inoculated with *E. coli* and held at 37°C.			
Coagulation	—	—	—	*	*	*	x	x
Reduction of litmus	*	*	*	*	—	—	—	*
Acid production	*	*	*	*	*	*	x	x
Peptonization	—	—	—	—	—	—	—	—
Controls	—	—	—	—	—	—	—	—

[1] Average of two determinations; incubated at 21 or 37°C. for 24, 48, 72, and 96 hours.
[2] (—) No reaction; (x) positive reaction; (*) partial reaction.

extent for this organism in each type of milk (Table 1-c). The biochemical reactions induced by *S. lactis* in soybean litmus milk were retarded as compared with the reactions obtained for litmus milk (Table 1-d). Acid production and coagulation of soybean litmus milk by *A. aerogenes* occurred within 24 hours at 37°C., whereas in litmus milk coagulation did not occur after 48 hours. Marked peptonization was produced by *A. aerogenes* in soybean milk, while in litmus milk peptonization occurred only slightly (Table 1-e). A partial coagulation and some acid formation occurred upon growth of *E. coli* in soybean milk; however, for litmus milk these reactions were only partial (Table 1-f).

Upon consideration of the entire incubation period range a slight increase in the hydrogen-ion concentration occurred in the soybean milk as produced by *L. bulgaricus, S. lactis, A. aerogenes, L. acidophilus,* and *S. liquefaciens;* whereas the pH value produced by *E. coli* in cow's milk was greater than that for soybean milk held under the same conditions (Table 2).

TABLE 2

A Comparison of pH on Cow's and Soybean Milk When Inoculated With L. bulgaricus, S. lactis, A. aerogenes, L. acidophilus, E. coli, and S. liquefaciens [1]

(a) Influence on pH of medium when inoculated with *L. bulgaricus* and held at 37°C.							
Type of milk	0 hr.	0 hr.	18 hr.	48 hr.	72 hr.	96 hr.	120 hr.
Soybean milk	6.70[2]	6.41[2]	4.80	3.85	3.59	3.50	3.51
Cow's milk	6.61	6.45	5.81	4.60	4.01	3.50	3.57
(b) *S. lactis* at 21°C.							
Soybean milk	6.61	6.45	5.61	4.13	4.21	4.50	4.50
Cow's milk	6.70	6.41	5.72	4.30	4.22	4.18	4.21
(c) *A. aerogenes* at 37°C.							
Soybean milk	6.70	6.41	5.21	5.00	4.97	4.95	5.01
Cow's milk	6.61	6.45	5.28	5.10	5.00	5.12	5.15
(d) *L. acidophilus* at 37°C.							
Soybean milk	6.70	6.41	4.21	3.60	3.50	3.58	3.61
Cow's milk	6.61	6.45	5.21	4.10	3.72	3.70	3.75
(e) *E. coli* at 37°C.							
Soybean milk	6.70	6.41	5.65	5.80	5.90	6.50	6.48
Cow's milk	6.61	6.45	5.02	5.10	5.10	5.05	4.95
(f) *S. liquefaciens* at 37°C.							
Soybean milk	6.70	6.41	5.30	4.58	4.48	4.52	4.55
Cow's milk	6.61	6.45	5.40	4.53	4.53	4.59	4.63

[1]Average of two determinations; samples incubated at 21 or 37°C. for 18, 48, 72, 96, and 120 hours. [2]Values given for the medium after sterilization.

All the test organisms that were observed micrometrically with the exception of *L. acidophilus* exhibited a uniformity of cell structure and size when grown in either cow's or soybean milk. The cell size of *L. acidophilus* grown in cow's milk was about one micron in diameter and one to 20 microns in length, whereas the cell size attained in soybean milk was approximately 1.25 microns in diameter and the length measured from two to 50 microns.

SUMMARY AND CONCLUSIONS

Samples of cow's and soybean milk were inoculated with *L. bulgaricus, L. acidophilus, S. liquefaciens, S. lactis, A. aerogenes,* and *E. coli.* A comparative study was made on the rate and extent of peptonization, acid production, coagulation, pH, and reduction of litmus produced by these organisms.

In some cases biochemical reactions were manifested more rapidly in soybean milk; however, variability in the production of these reactions occurred, some being prominent and others negligible. Growth of *L. bulgaricus* in soybean litmus milk showed that coagulation occurred more rapidly, whereas in litmus milk acid production and litmus reduction were more pronounced. *L. acidophilus* caused a more pronounced reduction of litmus in cow's milk, whereas the other biochemical reactions were similar in most instances. *S. liquefaciens* produced only a slight proteolysis in soybean milk, while in cow's milk pronounced proteolysis occurred. The biochemical reactions induced by *S. lactis* in soybean litmus milk were retarded as compared with the reactions in cow's milk. *A. aerogenes* produced extensive proteolysis in soybean milk and only a slight proteolysis in cow's milk. Cow's and soybean milk inoculated with *E. coli* showed a more rapid acid production and coagulation in soybean milk, only a slight reduction of litmus in both samples, and no peptonization.

All the organisms observed except *L. acidophilus* exhibited a uniformity of cell structure and size when grown in either cow's or soybean milk. The cell size of *L. acidophilus* in cow's milk averaged one micron in diameter and one to 20 microns in length, and for soybean milk the cell size averaged 1.25 microns in diameter and from two to 50 microns in length.

Soybean milk serves as an excellent culture medium for the propagation of the organisms studied.

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ANTIOXIDANT EFFECT OF SOYBEAN FLOUR
IN FROZEN PASTRY [1]

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There is a considerable and growing interest in the freezing and frozen storage of prepared food products. There is also a growing interest in the development of soybean products and their use in foods. In view of these trends, and because of the scarcity of published data dealing with the oxidation of fat in food mixtures at low temperatures, particularly in the presence of food material of a possible antioxidant nature, the present study was undertaken.

The antioxidant effect of soybean flour on purified substrates has been demonstrated. Hence this flour is a food material which might be expected to have antioxidant properties in a food mixture stored at low temperatures. Musher (1935) added raw soybean flour and bleached, extracted soybean flour to lard and found, using the Swift stability test, that both flours were effective antioxidants for lard. The raw flour was more effective than that which was bleached and extracted. Sylvester, Lampitt, and Ainsworth (1942) have presented data on the antioxidant effect on premier jus of soybean flour. They found that this flour had a stabilizing effect which was greater for fats having a long rather than a short initial induction period. They found also that the increase in the induction period was not proportional to the concentration of soybean flour, since a relatively smaller effect was obtained as the concentration of the flour was increased.

The constituents of soybean flour which behave as fat antioxidants are probably cephalin, the tocopherols, and other substances. The stabilizing effect of commercial soybean lecithin is probably due to its content of cephalin, Olcott and Mattill (1946). Both tocopherol and soybean lecithin have been shown to act as antioxidants for lard, but when used together, a considerably increased effect has been observed. The cephalin present in soybean lecithin acts as a synergist with tocopherol, Libby (1945) and Mattill (1945).

Antioxidants other than tocopherols and cephalin have been demonstrated in soybean flour. Thus, Dahle, and Nelson (1941) found that an alcohol extract and phospholipid fraction had antioxidant properties for butter. Hilditch and Paul (1939) presented evidence for yet other antioxidant substances. Some of these, according to Golumbic (1942, 1943), are similar to chromane-5,6-quinones.

The manufacturing process for most soybean flour involves heating of the material. Whereas this may inactivate the cephalin, thereby reducing the possible antioxidant effect of the flour, the heating process probably would destroy the soybean enzymes, peroxidase and lipase, which otherwise would no doubt accelerate oxidation and splitting of fat in a food mixture, Ball, Axelrod, and Kies (1943) and Lea (1939). That inactivation of the peroxidase is likely to occur during the manufacturing process, is shown by the work of Sumner and Tressler (1943) who investigated the enzyme activity of a number of types of soybean meal and found no peroxidase activity in most of those tested.

The common availability of soybean flour, together with the current interest in frozen storage of prepared foods, has suggested the desirability of obtaining information as to the effect of this flour on fat in a prepared food mixture held in frozen storage. Since such a study requires a parallel study of similar food mixtures containing no soybean flour, data relative to keeping quality of incorporated fat in non-soy mixtures under conditions of frozen storage would also be obtained.

That there is need for such information is indicated by statements recently appearing in papers by Greaves and Boggs (1945) and Hutchings and Evers (1946) dealing with problems in food preservation.

Though many food mixtures could not reasonably include soybean flour, certain fat-containing foods readily lend themselves to combinations with this flour.

In the present study a full-fat, debittered soybean flour was incorporated in a pastry mix.

EXPERIMENTAL PROCEDURE

In order to determine whether soybean flour might inhibit oxidation of the fat in pastry made with wheat flour, lard, salt, and water, and if so, whether a small or a comparatively large quantity would have greater effect, five sets of pastry were prepared, using 0, 5, 10, 15, and 20 per cent of full-fat soybean flour substituted for an equal weight of wheat flour in the formula. These pastries were rolled to the same thickness and divided into three parts; one part was stored raw, the second part was stored raw and baked when removed from the freezer, and the third part was baked before being frozen. All were held at —17.8°C.(0°F.) for periods up to six months. Peroxide numbers were determined on the fresh lard and on fat extracted from the pastry immediately after baking, and after three, four, and six months of frozen storage.

Pastry containing 20 per cent by weight of soybean flour substituted for wheat flour in the formula was compared with an all-wheat flour pastry in the study of keeping quality of fat in the crust of frozen pies. Pies were prepared having two different types of filling: green apple, an example of a fruit filling; and custard, an example of a non-fruit type.

Soy-crust pies and plain-crust pies, both apple and custard, were divided into three lots: Lot 1, baked before freezing; Lot 2, stored raw; Lot 3, stored raw, to be baked after removal from frozen storage. The peroxide values and free fatty acids were determined on the fresh lard, and on the fat extracted from the crusts of freshly baked pies of each kind. The pies to be stored in the freezing locker (0°F.) were made in

pie plates, frozen in the freezing compartment of a household-size refrigerator, removed from the plates, wrapped in heavy waxed paper, and stored for periods up to 12 months in the freezer locker.

The pies were stacked one on top of another, with no attention given to the position of the pies in the storage locker. This method of handling was necessary because of the type of locker and the amount of space available. There were inevitable variations in the protection or exposure of different pies to the atmosphere of the locker.

At intervals of two, four, eight, and twelve months, pies from each lot were removed from the locker. The pies which had been stored raw, to be baked after removal from the locker, were unwrapped, placed in pie plates, and allowed to thaw in the refrigerator overnight. They were then baked for the same length of time, and at the same temperature as were the pies baked prior to freezing.

Peroxide numbers and free fatty acids were determined on fat extracted from the pie crusts.

RESULTS AND DISCUSSION

The peroxide numbers of fat extracted from pastry containing 0, 5, 10, 15, and 20 per cent soybean flour substituted for an equal weight of wheat flour, stored at 0°F. for specified periods, are shown (Table 1).

In each series (i.e., raw pastry, pastry baked before storage, and pastry stored raw and baked after removal from storage) the fat extracted from

TABLE 1

Peroxide Numbers [1] of Fat Extracted From Pastries Stored at —17.8°C.(0°F.)

Storage period	Soybean flour	Raw pastry	Pastry baked before storing	Pastry stored raw; baked when removed from storage
mo.	*pct.*			
0	0	....	4.5	....
	5	....	2.0	....
	10	....	1.7	....
	15	....	1.5	....
	20	....	1.7	....
3	0	2.7	3.9	9.7
	5	1.4	1.6	2.3
	10	2.3	1.7	1.9
	15	3.4	2.2	1.5
	20	3.4 ·	2.2	1.7
4	0	2.3	3.7	10.5
	5	1.1̆	2.6	2.9
	10	1.1	2.6	3.2
	15	1.1	2.3	2.2
	20	1.6	4.3	2.2
6	0	2.3	3.4	11.0
	5	2.2	1.6	2.7
	10	1.2	2.0	1.8
	15	1.8	2.7	3.0
	20	3.1	1.2	2.0

[1] Me. H_2O_2 per 1,000 gm. of fat.

the pastry containing no soybean flour had higher peroxide values than did the fat from pastry containing soybean flour. In nearly every case, the peroxide values of fat extracted from pastry stored raw and baked after removal from storage were higher than were those for fat extracted from the corresponding raw pastry or pastry baked prior to storage. The protecting effect of the soybean flour appeared to be equally efficient at each of the levels studied.

Besides inhibiting fat oxidation, the soybean flour affected the tenderness of the pastry and the flavor and browning during baking. The effect on tenderness of substituting 5, 10, 15, or 20 per cent of soy flour for wheat flour in the pastry formula is shown by the following comparison of breaking strength of pastry wafers:

Soybean flour substituted for wheat flour (per cent)	Breaking strength (ounces)
0	20.6
5	20.6
10	19.1
15	16.0
20	14.4

As can be seen, the substitution of 15 or 20 per cent full-fat soybean flour for wheat flour in the formula markedly increased the tenderness of the pastry. The characteristic flavor of the soybean flour, though pleasant, was distinct at the 15- and 20-per cent levels, and would probably not be harmonious with the flavor of certain pie fillings.

The browning during baking of pastries containing soybean flour was more rapid, the higher the percentage of soybean flour incorporated. Since it was necessary for purposes of this study to maintain constant time and temperature during baking, a 20-per cent soybean substitution represented the highest level feasible for a desirable product.

Because of the increased tenderness of pastry made with 20 per cent substitution of soybean flour, and because this percentage appeared to be the maximum possible under conditions of this study, 20-per cent soybean pastry was selected to compare with an all-wheat flour pastry in the study of keeping quality of frozen pies.

The free fatty acid contents of fat extracted from wheat-flour crust are compared with those from soy-flour crust (Table 2). Each figure rep-

TABLE 2

Average Free Fatty Acid[1] Content of Fat Extracted From Plain and Soy Crust of Pies Stored at —17.8°C. (0°F.) up to 12 Months

Storage period	Wheat-flour crust	20% soy-flour crust
mo.		
Not stored		
(baked pies only)	1.1	1.2
2	1.3	1.4
4	1.5	1.6
8	1.5	1.5
12	1.4	1.6

[1] Mg. KOH per one gm. of fat.

resents the average of the free fatty acid values obtained on fat extracted from eight pie crusts, except the unstored baked pies, for which the figures given represent the average for three pie crusts.

The free fatty acids were low in fat extracted from pie crusts of all types. They showed slight variations but no marked trends. If lipase activity took place, it must have proceeded at a very slow rate.

A comparison of peroxide numbers of fat extracted from wheat-flour crusts and from 20-per cent soy-flour crusts for each period of frozen storage is shown (Table 3).

TABLE 3

Peroxide Numbers [1] of Fat Extracted From Crust of Pies Stored at −17.8°C.(0°F.)

Storage period	Wheat-flour crusts						
	Raw		Baked prior to freezing		Stored raw, baked when removed from freezer		Summary
	Apple	Custard	Apple	Custard	Apple	Custard	
mo.							
2	5.6	3.9	11.0	12.4	9.0	3.2	7.8
4	4.3	3.2	4.9	10.4	10.0	7.9	6.5
8	20.2	5.7	11.5	8.0	33.3	19.8	17.4
12	38.9	9.8	13.5	7.2	......	21.7	21.1
	Soy-flour crusts [2]						
2	4.8	3.6	3.8	2.0	3.5	2.9	3.4
4	1.2	3.3	2.8	1.7	5.6	1.2	3.0
8	3.1	2.4	4.4	6.9	4.7	7.8	4.6
12	3.1	2.9	3.3	1.9	9.4	3.1	4.4

[1] Me. H_2O_2 per 1,000 gm. of fat.   [2] Twenty per cent soy flour substituted for wheat flour.

The peroxide values of fat extracted from pie crust made with 20-per cent soybean flour were lower at each storage period than were those of fat extracted from corresponding pie crusts containing no soy flour.

Neither the raw nor baked custards were satisfactory. Custards which were baked, followed by freezing and thawing, had unsatisfactory texture. Preliminary tests did not result in satisfactory products even though the custards were but partially set when removed from the oven. The baked pies were stored and analyzed in order to supply some data in regard to non-fruit pies. The custard pies frozen raw and baked after removal from the locker were desirable as to consistency of the filling; but the flavor of the custard had noticeably deteriorated by the end of four months of storage.

Apple pies, whether stored raw and baked after removal from the locker or baked prior to freezing, retained a satisfactory flavor and texture throughout the 12-month storage period. Since peroxide numbers of fat extracted from the non-soy crusts of these pies were comparatively high after eight months of storage, it would probably be inadvisable to hold pies such as these under similar conditions of storage longer than eight months.

Evaluation by judging of the flavor and texture of prebaked apple pies was carried out after 14 to 15 months of frozen storage. One each, of the soy and plain apple pies baked prior to freezing were reheated at

home by four different families; one of each were reheated in the laboratory and an estimate of quality made by six students and teachers. No score card was provided. The families, students, and teachers were asked to make an estimate of the quality of the pies, and to say particularly whether they considered the pies good as compared with their standards for fresh green apple pie. The comments were that the pies were good to excellent. Opinions differed as to the comparative desirability of the soybean crust versus the plain crust. It was remarked that the soy crust, though very tender, was more crumbly than crisp. No sogginess of the crust was observed.

SUMMARY

The effectiveness of soybean flour as an antioxidant for lard in frozen pastry was tested, using pastry made with 0, 5, 10, 15, or 20 per cent full-fat, debittered soybean flour; and using pies of two different types, apple and custard, for which the pastry contained 0 or 20 per cent soybean flour. Three series of each type of pastry and pies were prepared. One series was baked prior to freezing; the second series was stored raw; and the third series was stored raw and baked after removal from the freezer.

Peroxide numbers were determined on fat extracted from pastry containing the different percentages of soybean flour. Free fatty acids as well as peroxide numbers were determined on fat extracted from crusts of pies held in frozen storage at 0°F. up to 12 months.

Free fatty acids were low and showed little difference between fats extracted from the different pie crusts.

In all cases, under conditions of this study, peroxide formation was inhibited in fat of pastry containing soybean flour. Peroxide numbers showed that fat in pastry containing soybean flour in proportions of 5, 10, 15, or 20 per cent kept about equally well, with little difference between soy pastry stored raw or that baked prior to freezing.

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RESAZURIN REDUCTION TEST AND MICROBIOLOGY OF EGG POWDERS PREPARED BY LYOPHILIZATION

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Methylene blue in the absence of oxygen is widely used to measure "reductase" activity of bacteria cells or of cell-free enzyme preparations. A modification of the "methylene blue technique," frequently called the "reductase test," has long been applied to milk containing dissolved O_2 as a means of estimating its sanitary history and keeping quality. The test is usually considered an indirect quantitative index of the viable bacterial content at the start of incubation. The substitution of resazurin for methylene blue was recommended by Pesch and Simmert (1928) and has since been used by many milk control laboratories.

Recently Scott and Gillespie (1943, 1944) applied the resazurin test to the quality grading of egg "pulp" prepared in various Australian states and found that the rate at which resazurin is reduced to pink color afforded a reliable index of the bacterial content of the pulp. Berry and Wolford (1945), using the technique described in this paper, examined stored frozen-egg emulsions and obtained reduction values approximating those of Scott and Gillespie. Johns (1944) investigated the usefulness of the reductase test in a study of the microbiology of egg powder using methylene blue and resazurin, and reported that the tests involved endpoint difficulties and long incubation periods.

Preliminary investigations on the application of the resazurin-dye test to inoculated egg liquid and powder indicated that the rate of dye reduction by inoculated lyophilized powders was not related to the plate count of such powders but appeared to be related to the number of viable bacteria in the melange prior to drying. The object of the work reported here, therefore, was to investigate the effect of dehydration by lyophilization and of storage on the rate of resazurin-dye reduction by inoculated and uninoculated egg emulsions in relation to their viable count.

METHODS

The eggs used were U. S. Grade A. The shells were broken aseptically by the method tentatively suggested for shell egg in "Standard Methods for the Examination of Dairy Products" (1941). Half of each lot of egg emulsion was inoculated with *Pseudomonas fluorescens*.[3] This organism was

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chosen for inoculation, as members of this genus occur with relative frequency in commercial egg liquids. To emphasize any difference between powders of good sanitary history (uninoculated) and poor sanitary history (inoculated) massive doses of *Ps. fluorescens* were used. The inoculated emulsions before lyophilization contained from 430 x 10^6 to 3700 x 10^6 viable organisms per gram of egg liquid. However, the plate counts of the stored powders prepared from inoculated liquids were comparable to those frequently reported for commercially dried eggs.

The inoculated and uninoculated liquids were churned in sterile Waring blendors, equipped with glass containers, and distributed into 500-ml. boiling flasks in 100-ml. quantities. After distribution the liquids were stored overnight at 2.2°C.(36°F.). The following morning they were shaken vigorously and dried by vacuum from the frozen state (lyophilized) to powders containing two per cent moisture or less. This method of drying permitted adequate quality control of the raw material and essentially aseptic conditions during processing. The lyophilized powders were transferred to sterile, screw-cap, glass containers and sealed with paraffin for storage.

Plate counts were made on emulsions prepared by mixing five grams of shell egg liquid, or two to five grams of egg powder as available, with sterile, distilled water according to tentative procedures described by the American Public Health Association in "Standard Methods for the Examination of Dairy Products" (1941). One-ml. aliquots of appropriate dilutions were plated in duplicate in tryptone-glucose-extract agar, and incubated at 30°C.(86°F.) for three days. Direct counts were made by spreading 0.01 ml. of 1:10 dilution of shelled-egg liquid, or 1:40 dilution of emulsified egg powder, over a one-sq. cm. area previously marked on a clean glass slide. Duplicate preparations were made. The films were defatted and fixed by two one-minute treatments in xylene and two one-minute treatments in absolute methanol. The preparations were stained with a 2:1 mixture of methylene blue and basic fuchsin, as described by Gray (1943) for the direct count of bacteria in milk. Since emulsified or reconstituted whole-egg powders are alkaline, it was found necessary to acidify with 0.5 N HCl to approximately pH 5.5 before staining. The bacterial count was facilitated by the use of a ruled ocular micrometer disc giving a field which measured 0.1 x 0.1 mm. A factor of 1,000,000 was therefore used to obtain the number of bacteria in 1.0 ml. of dilution examined.

Moisture determinations on the egg powders were made according to the procedure adopted by the Association of Official Agricultural Chemists (1940) for moisture in dried eggs.

For convenience of application to both liquid-egg and powdered-egg samples, a slight modification of the usual "reductase" test was employed: 4.0 ml. of liquid whole egg emulsified with 6.0 ml. of sterile, distilled H_2O, or 1.0 gram of dry egg powder emulsified with 9.0 ml. of sterile, distilled H_2O, were placed in sterile 18 x 150-mm. test tubes and 0.2 ml. of 0.05 per cent resazurin dye solution added. The egg-pulp concentration was

40 per cent of that used by Scott and Gillespie (1943, 1944) and corre-
sponded to 1:10 dilution of powder. This concentration of powder was
convenient for plating; i.e., 10-ml. aliquots could be withdrawn from the
first dilution bottle prepared for the plate counts. The tubes were stop-
pered and inverted several times to insure thorough mixing, stoppers were
replaced by sterile cotton plugs, and tubes were placed in a 35°C.(95°F.)
water bath protected from light. At each observation time during the
first stage of dye reduction (blue to pink) the tubes were gently shaken.
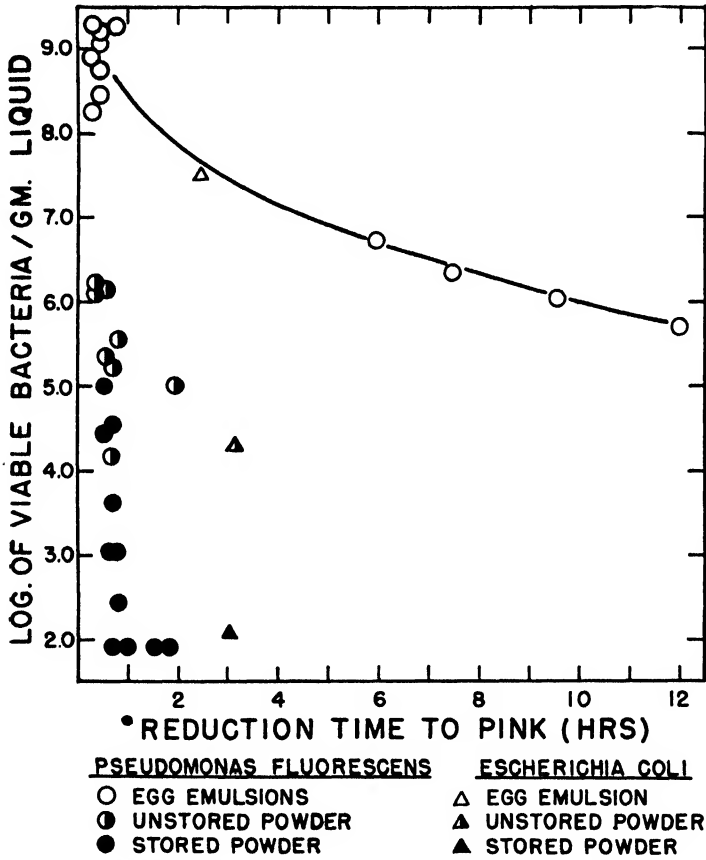
Fig. 1. Effect of lyophilization and storage on relationship of resazurin reduction to
plate counts of inoculated eggs.

During the second stage (pink to colorless) care was taken not to shake
the contents. Color change to pink was measured ·by comparison with plate
No. 43 in the book by Maerz and Paul (1930). A glass plate with slight
yellow tinge was placed over the color chart and served to improve color
comparisons. Variations in color of egg emulsions, in light (whether natu-
ral or artificial), and in dye lots affected color comparisons. The dye
solution was sterilized in the autoclave at 10 pounds' steam pressure for
10 minutes before use.

EXPERIMENTS

Resazurin Reduction by Inoculated and Uninoculated Emulsions Prepared From Shell Eggs: To determine whether resazurin reduction by lyophilized egg powders would serve as a criterion of bacterial (*Pseudomonas fluorescens*) contamination, it was necessary to (a) determine the rate of resazurin reduction by fresh, uninoculated (sterile) egg liquid and (b) establish quantitative relationships between dye-reducing power and numbers of viable *Ps. fluorescens* in inoculated egg liquid. Using the technique described, it was found that sterile egg liquid did not change the dye color further than blue-lavender in 24 hours. Addition of increasing amounts of egg liquid containing 70 x 10^5 viable *Ps. fluorescens* organisms per milliliter caused a progressive increase in resazurin reduction rate (Fig. 1).

TABLE 1

Effect of Lyophilization on Resazurin Reduction by Whole-Egg Emulsions, Uninoculated and Inoculated With Ps. fluorescens

History	Before lyophilization			After lyophilization		
	Plate [1] count	Direct count	Time for reduction to pink	Plate count	Direct count	Time for reduction to pink
	$x10^6$	$x10^6$	*min.*	$x10^6$	$x10^6$	*min.*
Inoculated........................	760	3700	30	0.21	3200	45
Inoculated........................	430	3600	30	0.38	3600	35
Inoculated........................	3700	2500	50	0.55	1800	60
Inoculated........................	240	8300	20	2.30	5100	25
Inoculated........................	990	8100	20	1.60	4600	35
Inoculated........................	2100	2000	30	0.12	1800	120 [2]
Inoculated........................	3100	5100	<30	1.40	3400	30
Inoculated........................	1400	4900	30	0.02	3100	45
Uninoculated.....................	<0.0001	....	Little change in 5-8 hours	<0.0001	....	Little change in 5-8 hours
Uninoculated.....................	<0.0001	....		<0.0001	....	
Uninoculated.....................	<0.0001	....		<0.0001	....	
Uninoculated.....................	<0.0001	....		<0.0001	....	

[1] All plate and direct counts are given as number of bacteria per gram of whole-egg liquid. As lyophilization reduced whole-egg liquid to approximately one-fourth its original weight, the numbers of bacteria per gram of powder are divided by 4. [2] Owing to breakdown in apparatus, frozen emulsion melted before drying was completed.

Effect of Lyophilization on Resazurin Reduction by Inoculated and Uninoculated Egg Emulsions: Throughout a six months' period, eight lots of egg liquid were heavily inoculated with *Ps. fluorescens,* lyophilized, and tested for resazurin-reducing action and viable count. The results show that over 99 per cent of the bacteria failed to grow after dehydration, but that dye reduction by the emulsions prepared from inoculated powders remained relatively high. The rate of dye reduction by the emulsions of inoculated powders showed a general correlation with the direct counts and with the numbers of viable organisms in the liquid before drying, but bore little or no relationship to the plate counts after drying. The dye reduction by emulsions prepared from uninoculated egg liquid and powder was negligible compared with the reducing action of the emulsions from inoculated powders (Table 1). Some loss of dye-reducing power occurred during lyophilization, but this was not proportional to the loss of viable

TABLE 2

Effect of Storage on Resazurin Reduction of Egg Powders Prepared From Inoculated and Uninoculated Emulsions

History	Before storage					After storage				
	Plate[1] count $\times 10^6$	Direct[2] count $\times 10^4$	Time for reduction to: Pink min.	Colorless hr.		Storage time and temperature	Plate[1] count $\times 10^6$	Direct[2] count $\times 10^5$	Time for reduction to: Pink min.	Colorless hr.
Inoculated Ps. fluorescens...	0.21	3200	45	...		2 days at 36°F.	0.057	4000	45	...
						1 month at 36°F.	<0.0001	......	120	...
Inoculated Ps. fluorescens...	0.38	3600	35	...		2 days at 36°F.	0.10	......	35	...
Uninoculated..............	<0.0001	......	No change in 5 hr.			2 days at 36°F.	<0.0001	......	No change in 5 hr.	
						1 month at 36°F.	<0.0001	......	No change in 5 hr.	
Inoculated Ps. fluorescens (a)	2.30	5100	20	...		Composite (a)+(b)	0.002	6300	40	...
(b)	1.60	4600	20	...		9 days at 36°F.			...	...
Uninoculated..............	<0.0001	......	No change in 8 hr.			9 days at 36°F.	<0.0001	......	No change in 8 hr.	
Inoculated Ps. fluorescens...	1.40	3400	30	3.5		2 weeks at −20°F.[3]	0.048	......	35	>3
						4 weeks at −20°F.	0.006	......	45	4
						8 weeks at −20°F.	0.001	......	45[4]	>5
						12 weeks at −20°F.	<0.0001	2300	60	<24
Inoculated Ps. fluorescens...	1.40	3400	30	3.5		2 weeks at 9.5°F.[3]	<0.0005	......	50	>24
						4 weeks at 9.5°F.	<0.0001	......	45	>24
						8 weeks at 9.5°F.	<0.0001	......	45[4]	>48
						12 weeks at 9.5°F.	<0.0001	2400	60	>48
Inoculated Ps. fluorescens...	0.02	3100	4.5	3.5		12 weeks at −20°F.	<0.0001	2000	90	24
						12 weeks at 9.5°F.	<0.0001	1900	120	48
Uninoculated..............	<0.0001		No change in 5 hr., blue-gray (B2) in 24 hr.			2 weeks at −20°F.[3]	<0.0001	......	No change in 5 hr. blue-gray (B2-3) in 24 hr.	
						4 weeks at −20°F.	<0.0001	......	No apprec. change in 5 hr.; blue-lavender (C3) in 24 hr.	
						8 weeks at −20°F.	<0.0001	......	No apprec. change in 5 hr.;[4] blue-lavender (C3-4) in 24 hr.	
						12 weeks at −20°F.	<0.0001	......	No apprec. change in 5 hr.; blue-lavender (C3-4) in 24 hr.	
Uninoculated..............	<0.0001		No change in 5 hr., blue-gray (B2) in 24 hr.			2 weeks at 9.5°F.[3]	<0.0001	......	Blue-lavender (B-C3) in 4 hr.; lavender-pink (D-E2) in 24 hr.	
						4 weeks at 9.5°F.	<0.0001	......	Blue-lavender (C3) in 4 hr.; pink-lavender (E1-2) in 24 hr.	
						8 weeks at 9.5°F.	<0.0001	......	Blue-lavender (C2) in 4 hr.; pink-lavender (F1-2) in 24 hr.	
						12 weeks at 9.5°F.	<0.0001	......	Blue-lavender (C2) in 4 hr.; pink (G1-2) in 24 hr.	

[1] See footnote 1, Table 1. [2] Direct count approximates original inoculum of liquid emulsions. [3] Before storage the inoculated and uninoculated powders contained 2.0 and 1.9 per cent moisture, respectively. After distribution, sampling, and three months' storage, the powders in the above order contained 3.0, 3.4, 3.7, and 3.2 per cent moisture, respectively. [4] These samples were also run in Thunberg tubes, with resazurin, under nitrogen. No differences could be detected in results up to five hours.

Ps. fluorescens cells (Fig. 1). One lot of eggs heavily inoculated with *Escherichia coli* and tested before and after lyophilization gave similar results (Fig. 1).

Retention of Resazurin-Reducing Action by Emulsions Prepared From Inoculated and Uninoculated Egg Powders During Storage: Inoculated and uninoculated egg liquids were lyophilized and stored at 2.2°C.(36°F.) for four weeks and —28.9 and 35°C.(—20 and 95°F.) for 12 weeks. Samples were removed during storage for plate counts and for dye-reduction determinations. The results show that, in general, viable counts of the stored inoculated powders decreased rapidly, but that the dye-reducing action of these powders, as determined by first stage of dye reduction (blue to pink) remained relatively high (Table 2). Dye reduction by emulsions of uninoculated powders increased with increasing storage time at 95°F., but within the conditions of this experiment remained low or negligible as compared with the activity of emulsions of inoculated powders.

Decrease in the dye-reducing action, attributable to bacteria, occurred during storage. Activity losses were greater at 95°F. than at —20°F. These losses were not so apparent in observations of the first stage of resazurin reduction (blue to pink) but were apparent in observations of the second stage of dye reduction (pink to colorless). The reason for this is not known but is illustrated by comparing the first and second stage reduction times of the emulsions of stored inoculated powders (Table 2). The dye-reducing power (bacterial) of the inoculated stored powders (as determined by the blue-to-pink stage) bore little or no relationship to the number of viable *Ps. fluorescens* cells (Fig. 1).

Effect of Heat (149°F. for 20 Minutes) on Resazurin Reduction by Inoculated and Uninoculated Egg Emulsions: Three lots of uninoculated, whole-shell-egg liquids and three lots of the same liquids inoculated with *Ps. fluorescens* were maintained at 65°C.(149°F.) for 20 minutes. The preheating time to bring to this temperature was approximately 20 minutes. Plate counts and dye-reduction tests were made before and after heating. The results indicate the thermolability of the dye-reducing action attributable to bacteria under the conditions of this experiment. After heating, over 99.9 per cent of the *Ps. fluorescens* organisms failed to grow and losses of approximately 90 per cent of reducing activity were observed (Table 3). Dye reduction by the uninoculated, heated emulsions remained low or negligible compared with that by the inoculated emulsions.

Resazurin-Reducing Action of Various Bacteria in Shell-Egg Emulsions: It is, of course, recognized that organisms differ in resazurin-reducing power. This is illustrated in liquid egg by pure-culture study. Using similar levels of inoculum in sterile-egg emulsions, it was found that two strains of *Escherichia coli, Micrococcus conglomeratus,* and an unidentified bacterium isolated from a commercially dried egg powder reduced resazurin to colorless within five hours, but that *Bacillus mesentericus, B. subtilis,* and *Lactobacillus casei* caused no color change during this time. Emulsions heavily inoculated with *Micrococcus lysodeikticus,* which is, of course, lysed by egg lysozyme, caused dye reduction to pink within one hour when tested immediately after the addition of the bacteria.

TABLE 3

Effect of Heat (149°F. for 20 Minutes) on Resazurin Reduction by Whole-Egg Emulsions, Uninoculated and Inoculated With Ps. fluorescens

Sample No.	History	Before heating		After heating	
		Plate count[1] $x10^6$	Dye reduction	Plate count[1] $x10^4$	Dye reduction
1 a........	Inoculated	1090	Pink 20 minutes; colorless, 3 hr.	0.11	Pink, 24 hr.
b........	Uninoculated	<0.0001	Blue-lavender, 24 hr.	<0.0001	Blue-lavender, 24 hr.
2 a........	Inoculated	1140	Pink, 20 min.	0.0002	Lavender-pink, 24 hr.
b........	Uninoculated	<0.0001	Blue-lavender, 24 hr.	<0.0001	Blue-lavender, 24 hr.
3 a........	Inoculated	1020	Pink, 20 min.	0.0005	Lavender-pink, 24 hr.
b........	Uninoculated	<0.0001	Blue-lavender, 24 hr.	<0.0001	Blue-lavender, 24 hr.

DISCUSSION

If dye-reduction rates were related to the number of viable organisms, a sharp decrease in the number of viable bacteria should cause corresponding decreases in rate. This study shows, however, that the general correlation existing between plate count and rate of resazurin reduction by liquid eggs inoculated with *Ps. fluorescens* may be disrupted by lyophilization and further by storage. Since dye reduction by emulsions prepared from inoculated powders bears little or no relationship to the plate counts of the powders, the rate could not be related to the number of viable organisms present at the start of the incubation period of the "reductase" test, and indicates that bacterial catalysts (enzymes), or reducing substances formed by the bacteria before or after the death of the organisms, were responsible for resazurin reduction. The relatively high reducing powers of the inoculated, lyophilized powders showed that these catalysts, or the reducing substances, remained active after the death of the organisms but did not prove that these agents contributed to quality changes in the powder during storage. Since emulsions prepared from uninoculated egg powders caused a slow dye-color change, and since storage of the powder at 95°F. increased the rate of color change, it appears possible that chemical reactions between egg components may cause resazurin color change. The role of bacterial catalysts or reducing agents and the influence of egg components on resazurin reduction in egg power will be the subject of a further communication.

The study suggests that the sanitary history of certain foods not subjected to heat treatment in the process of manufacture might be gauged by the resazurin test.

SUMMARY AND CONCLUSIONS

The resazurin dye-reduction test was applied to uninoculated whole-egg emulsions, to emulsions inoculated with *Ps. fluorescens*, and to the powdered products prepared by lyophilization. It was found that a general relationship existed between plate count of liquid eggs inoculated with *Ps. fluorescens* and dye-reducing time. This relationship was disrupted by lyophilization and further by storage. The resazurin-reducing action of emulsions prepared from inoculated powders remained relatively high, although plate counts were reduced 99 per cent or more by lyophilization and decreased to lower and negligible values during storage. Losses during storage of dye-reducing power having bacterial origin, were greater at 95°F. than at —20°F., as indicated by the second stage of dye reduction in tests of emulsions prepared from the stored inoculated powders. These losses were not so apparent when measured by the first stage of dye reduction.

When egg liquid, inoculated with *Ps. fluorescens*, was heated at 149°F. for 20 minutes, it was found that approximately 99.9 per cent of the bacteria failed to grow after heating and that losses of over 90 per cent of dye-reducing activity also occurred.

Sterile-egg emulsions inoculated with similar levels of two strains of *Escherichia coli, Micrococcus conglomeratus,* and an unidentified bacterium isolated from commercially dried egg powder caused resazurin reduction

within five hours, while like inoculations of *Bacillus mesentericus, B. subtilis,* and *Lactobacillus casei* did not cause color change during this time. A heavy inoculation with *Micrococcus lysodeikticus,* which is sensitive to lysozyme, caused dye reduction when tested immediately after addition of the organism.

Dye-reducing action by uninoculated egg liquid and by emulsions prepared from uninoculated, freshly lyophilized powder was low or negligible compared with inoculated liquids. The reducing action of the uninoculated liquid egg heated at 149°F. for 20 minutes was also low. Dye-reducing action by emulsions prepared from uninoculated stored powders increased with storage time at 95°F., but remained low compared with that by emulsions of inoculated powders.

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EFFECT OF BACTERIA ON QUALITY OF STORED LYOPHILIZED EGG POWDERS

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Little work appears to have been done on the influence of bacteria on the loss of powder quality as determined by the usual quality tests of palatability, fluorescence, solubility, etc. DeBord (1925) noted that a somewhat rancid odor developed in powders during storage, particularly when those powders were prepared from low-grade eggs and stored at 37°C.(98.6°F.). He stated that the counts of viable bacteria furnished little basis for estimating the quality of the product. Thistle, Pearce, and Gibbons (1943) found that moisture content and bacterial plate count are independent measures of quality, and that the slight degree of association between bacterial count and palatability in prime-quality powders was presumably fortuitous. Gibbons and Fulton (1943) stated: "Although there is as yet no direct evidence that any correlation exists between bacterial content and quality of egg powders, the number of bacteria gives some indication of sanitation in the . . . processing plants." Recently Stuart, Goresline, Smart, and Dawson (1945) showed that spray-dried egg powder of low sanitary quality decreased in solubility during storage to a greater extent than powder of high sanitary quality.

Marked differences in deteriorative changes during storage, as measured by the usual quality criteria, in commercially spray-dried and in lyophilized whole-egg powders have been observed in this laboratory. Some of the differences in storage behavior were quite probably related to differences in drying procedures and some to the quality of the shell egg used. It is obvious that microbial growth in the egg liquid might cause undesirable changes which would affect the quality of the powdered product. It is reported in a companion communication by Hirschmann and Lightbody (1947) that catalysts or reducing substances of microbial origin survived lyophilization and retained the power to reduce resazurin dye. It appeared possible that these agents might accelerate changes and shorten storage life of egg powder. The object of this study, therefore, was to determine whether large numbers of *Pseudomonas fluorescens*, incorporated in egg liquids prior to drying by lyophilization, contributed to deterioration of the low-moisture powders during storage, as estimated by the usual criteria of quality.

METHODS

Liquid egg pulp was collected aseptically and one half of each lot inoculated with *Ps. fluorescens*. The liquids were dried by lyophilization, and the powders stored in sealed glass containers. Details concerning inoculum, distribution, lyophilization, and storage and the methods used for moisture

determinations and plate counts are described in the companion communication. Hydrogen-ion concentrations were determined on 1:10 dilutions of the powder in distilled water. Free fatty acid content was determined by the procedure recommended by Kline and Johnson (1946). For palatability values the reconstituted powder was mixed with equal parts of fresh shell egg and cooked over water at 87.8°C.(190°F.) with stirring until coagulated. The flavor of the cooked eggs was rated in duplicate by eight tasters and the average of all scores reported.

Salt-soluble fluorescence was determined by the method used by Pearce, Thistle, and Reid (1943). Lipid fluorescence, described by Dutton and Edwards (1946), was determined on a Coleman fluorometer standardized for quinine sulfate, as described by these authors. In order to conserve material and labor, the three chloroform extracts obtained from the sample prepared for the salt-soluble fluorescence determination were combined and brought to 100-ml. volume by addition of c.p. chloroform. For the fluorometric reading, five ml. of this extract was further diluted with 20 ml. of chloroform. The fluorometer was corrected for the fluorescing value of the chloroform.

A modification of the resazurin-reducing test, using dry dye, was employed as a means of following changes in the inoculated and uninoculated egg powders during storage. Sterile resazurin dye (equivalent to 5.0 ml. of 0.05 per cent resazurin solution per 100 ml. of egg pulp) was added to the egg liquid immediately before freezing for lyophilization. The resulting colored powders were observed for dye reduction during storage, reduction being indicated by changes in color which progressed from green to blue, lavender, pink, and "fading." As no satisfactory chart was available for the entire color range, subjective observations were compared with controls kept at —28.9°C.(—20°F.). The blue to lavender-pink range was compared with Maerz and Paul's color charts 42 and 43 in *A Dictionary of Color* (1930). A glass plate was placed over the charts, which imparted a slight yellowish tinge to the colors and improved the comparisons.

EXPERIMENTS

Effect of Ps. fluorescens on Stored Lyophilized Eggs as Determined by Salt and Lipid Fluorescence: Liquid eggs, uninoculated and inoculated with *Ps. fluorescens,* were lyophilized to powders containing 1.9 and 2.0 per cent moisture. Portions of the powders were stored at —28.9 and 35°C. (—20 and 95°F.) in sealed containers. Plate counts were made on the egg liquids and powders before storage and after two, four, eight, and 12 weeks of storage. The fluorescence values of the salt-water extracts and chloroform extracts of the powders were determined at the end of the storage periods.

Over 99.9 per cent of the *Ps. fluorescens* failed to grow after lyophilization when the numbers of viable organisms were calculated on the basis of cells per gram of liquid egg. The death rate of the surviving organisms was very high during storage time. At 95°F., over 99 per cent of the remaining organisms failed to grow after two weeks' storage, and a comparable loss of viable organisms occurred at —20°F. after 12 weeks' storage (Fig. 1).

The results of the fluorescence studies show that the addition of *Ps. fluorescens* caused an increase in salt-soluble fluorescence before storage and an increase in lipid fluorescence during storage at 95°F. (Table 1). The difference in the initial salt-soluble fluorescence values of the inoculated and uninoculated powders was maintained during storage (Fig. 2) and indicated that the cell bodies contributed to the salt-soluble fluorescence. The apparent increase in salt-soluble fluorescence in the inoculated powder at eight weeks may fall within the limits of experimental error. The incorporation of *Ps. fluorescens* did not increase lipid fluorescence of the powders before storage nor did it increase the lipid fluorescence values during 12 weeks' storage at —20°F. However, the inoculated powders stored at 95°F. showed an increase in lipid fluorescence above that of the uninoculated powders stored under the same conditions (Fig. 3). Thus the lipid fluorescence indicates changes during storage attributable to the bacteria or to their components which remained chemically active after death of the organisms.

Effect of Ps. fluorescens on Stored Lyophilized Eggs as Determined by Using Resazurin Dye Incorporated in the Powder: The results obtained by applying the resazurin "reductase" test to 1:10 dilutions of powders prepared from inoculated and uninoculated egg liquids are reported in a companion communication. It was found that (1) emulsions prepared from uninoculated egg powders reduce the dye relatively slowly (a measureable change in the blue-violet range occurred in 24 hours); (2) storage of the uninoculated powders slightly increased dye reduction rate by the emulsions prepared from such powders; and (3) emulsions of inoculated egg powders, tested before or during storage, reduced resazurin to pink at a rate which bore a general relationship to the original number of *Ps. fluorescens* cells in the liquid before lyophilization. This latter finding showed that the dye-reducing factor (or factors) of bacterial origin was present in stored powders, but it did not prove that the factor (or factors) contributed to quality changes in the powders during storage.

In the present study uninoculated liquid eggs and liquid eggs inoculated with *Ps. fluorescens* were prepared as described by Hirschmann and Lightbody (1947), and sterile resazurin solution was added. The liquids, with added dye, were dried by lyophilization to colored powders containing 1.9 and 2.2 per cent moisture, respectively.[3]

A portion of each powder was equilibrated to contain 7.2 per cent moisture. The powders were then stored at 4.4, 25, 30, and 35°C.(40, 77, 86, and 95° F.) in the dark for four weeks, and changes in their colors were observed. The results are given (Fig. 4).

Because of color changes that took place in the inoculated liquid eggs during mixing preparatory to freezing for drying, the initial colors of the inoculated powders were blue-gray and of the uninoculated powders, green-gray. In those preparations stored at temperatures sufficiently low that subsequent changes took place slowly, the rates of color changes were comparable. It was therefore assumed that damages before drying did not

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[3] Some moisture was absorbed during distribution, sampling, and storage of the powders.
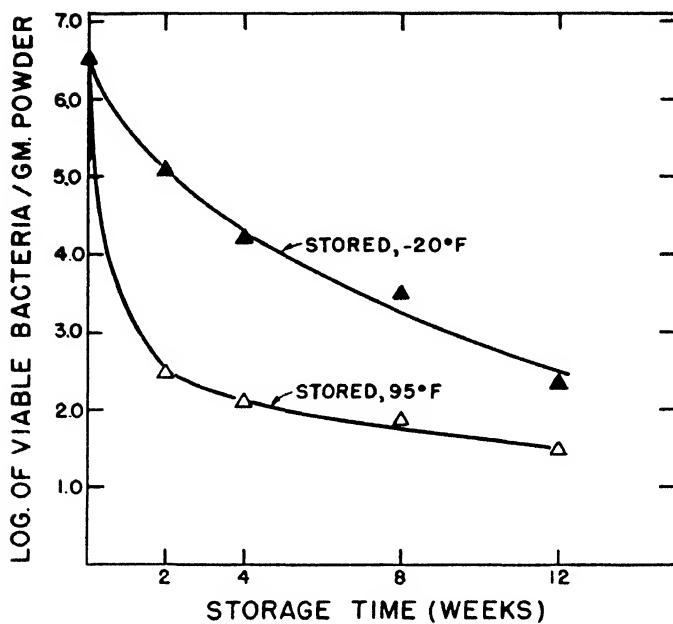
FIG. 1. Death rates of *Pseudomonas fluorescens* in stored lyophilized eggs.
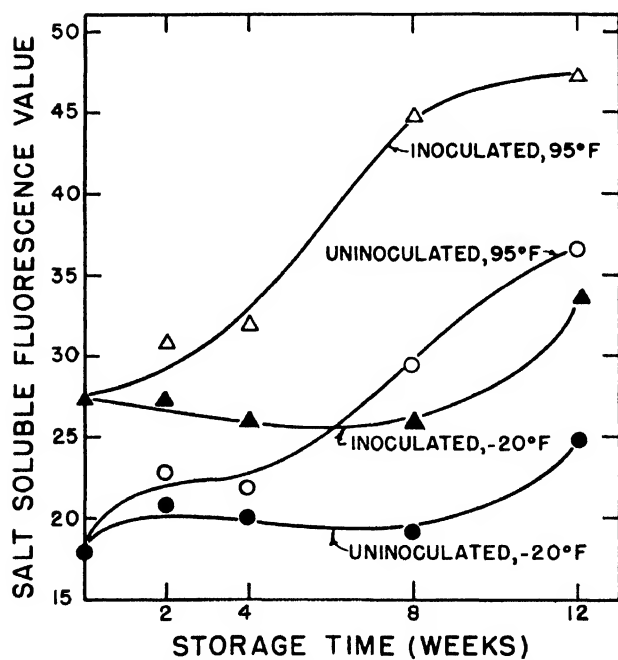
FIG. 2. Effect of *Pseudomonas fluorescens* on salt soluble fluorescence of lyophilized eggs.

TABLE 1

Effect of Pseudomonas fluorescens Incorporated in Lyophilized Egg Powder on Salt and Lipid Fluorescence During Storage at —20 and 95°F.

Powder history	Quality test of lyophilized powder	Storage period (weeks)[1]				
		0	2	4	8	12
Inoculated 31 x 10^9 viable *Ps. fluorescens* per gm. liquid stored —20°F.	Plate count (per gm. powder)	55 x 10^5	19 x 10^4	25 x 10^3	5300	390
	Salt fluorescence	27.5 / 27.5	27.8 / 27.8	26.2 / 26.2	25.0 / 28.0	34.0 / 34.0
	Lipid fluorescence	4.0 / 4.0	4.0 / 3.75	4.0 / 4.0	4.0 / 4.75	5.0 / 5.0
Inoculated 31 x 10^9 viable *Ps. fluorescens* per gm. liquid stored at 95°F.	Plate count (per gm. powder)	55 x 10^5	500	<100	<100	<100
	Salt fluorescence	27.5 / 27.5	31.0 / 31.0	32.0 / 32.0	45 / 44.5	47.6 / ....
	Lipid fluorescence	4.0 / 4.0	16.0 / 15.0	30.0 / 29.0	50.0 / 50.0	53.0 / 54.0
Uninoculated <100 viable bacteria per gm. liquid stored —20°F.	Plate count (per gm. powder)	<100	<100	<100	<100	<100
	Salt fluorescence	19.5 / 17.3	21.5 / 21.3	20.0 / 20.0	17.8 / 18.2	25.0 / 25.0
	Lipid fluorescence	4.0 / 4.0	4.0 / 4.0	4.0 / 4.0	4.75 / 5.0	5.0 / 5.0
Uninoculated <100 viable bacteria per gm. liquid stored 95°F.	Plate count (per gm. powder)	<100	<100	<100	<100	<100
	Salt fluorescence	19.5 / 17.3	23.4 / 23.0	22.9 / 22.9	29.5 / 29.5	37.5 / ....
	Lipid fluorescence	4.0 / 4.0	8.0 / 8.0	18.5 / 19.0	38.0 / 38.0	48.0 / 50.0

[1] Before storage the inoculated and uninoculated powders contained 2.0 and 1.9 per cent moisture, respectively. After distribution, sampling, and three months' storage, the powders in the above order contained 3.0, 3.4, 3.7, and 3.2 per cent moisture, respectively. At the end of the storage period, the pH of the powders in the same order were 7.4, 6.5, 8.0, and 7.8.

influence the rate of color change during storage. It was found that both inoculated and uninoculated dried eggs stored at each of the two moisture levels at 95°F. showed marked reducing properties as measured by resazurin color changes. It is also evident that rates of reduction at 95, 86, and 77°F. were greater in the powders of higher moisture content. It was also found that inoculated powders increased the rate of color change above that shown by uninoculated powders.
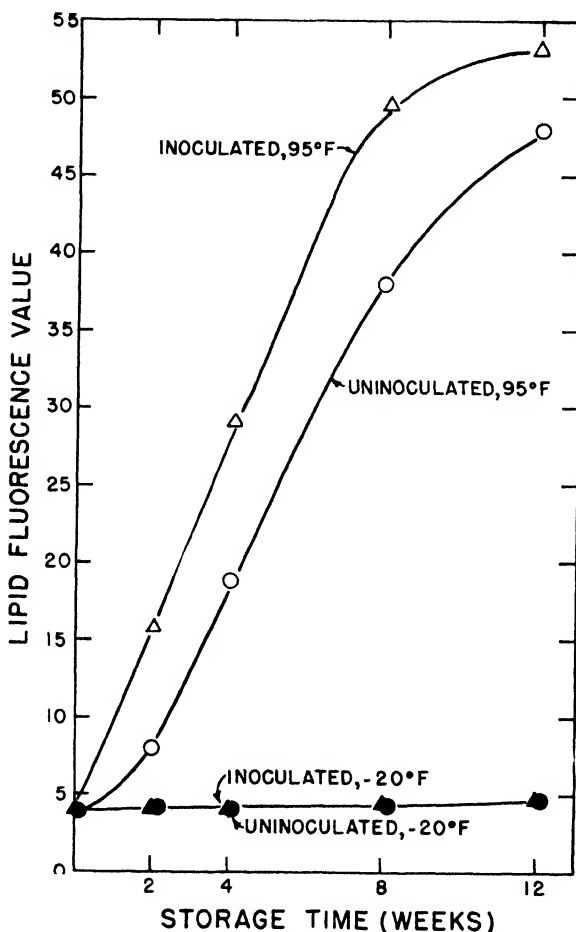
FIG. 3. Effect of *Pseudomonas fluorescens* on lipid fluorescence of lyophilized eggs.

Effect of Ps. fluorescens on Lyophilized Egg Powders Stored 12 Weeks as Determined by Various Quality Tests: Other lots of uninoculated and *Ps. fluorescens*-inoculated egg liquids were prepared; resazurin dye solution was added; and the liquids were immediately and rapidly frozen.[4] In this

[4] In order to reduce the initial color changes to a minimum, the time elapsing between addition of dye to egg liquid and placement in the freezing mixture was shortened to less than five seconds.

case the lyophilized powders contained 1.9 and 1.8 per cent moisture, respectively. Moisture determinations, plate counts, and salt-soluble and lipid-fluorescence tests were made on the powders before storage. The powders were stored at —20 and 95°F. in the dark for 12 weeks. Dye-color changes were observed during storage, and the following tests were made at the end of the storage period: moisture content, plate count, salt-soluble and lipid fluorescence, pH, free fatty acid content, and palatability. The results are summarized (Table 2).
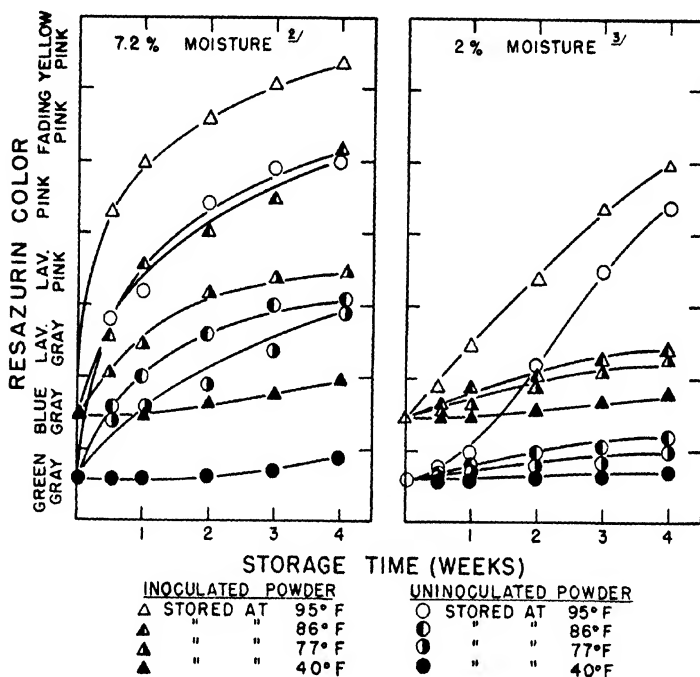
Fig. 4. Effect of *Pseudomonas fluorescens* on resazurin reduction [1] by lyophilized eggs.

[1] The dye was added to the emulsion before lyophilization, and color changes occurred after drying and equilibration.

[2] The powders containing 7.2 per cent moisture were obtained by allowing the freshly lyophilized powders to attain equilibrium in air-free desiccators containing sulphuric acid solution according to the method used by Makower (1945) for dehydrated eggs.

[3] The powders containing two per cent moisture adsorbed moisture during distribution and storage. After six and one-half months' storage, the powders averaged 5.7 per cent moisture.

It was found that *Ps. fluorescens* (or components remaining active following the death of the organisms) contributed to undesirable changes in egg powders stored at 95°F. for 12 weeks, as estimated by increases in free fatty acid content, lipid fluorescence, and rate of dye-color change. It was also found that the stored inoculated powders had lower pH values and palatability scores [5] than the corresponding uninoculated powders.

[5] Numerous palatability tests made in this laboratory have failed to detect changes of palatability of samples stored at —20°F. for many months.

TABLE 2

Effect of Pseudomonas fluorscens on Quality of Lyophilized Egg Powder Stored Three Months at —20 and 95°F.

Powder history	Storage	Moisture content	Plate count (per gm. Powder)	Resazurin powder color	Quality tests				
		pct.			Salt fluorescence	Lipid fluorescence	pH	Free fatty acid (mg. oleic/gm.)	Palatability[1] score
Inoculated Prepared from liquid egg containing 14 x 10⁸ viable Ps. fluorescens per gram of liquid	Before storage	1.8	76 x 10³	Green-gray	24.8 25.0	4.0 4.0	.....	.....	.....
	3 months' storage at —20°F.	2.4	260	Blue gray	20.1 20.1	4.75 5.0	7.5	5.5	6.4
	3 months' storage at 95°F.	2.4	<100	Pink-gray	33.0	56.0 56.0	6.6	49.3	4.2
Uninoculated Prepared from liquid egg containing <100 viable bacteria per gram of liquid	Before storage	1.9	<100	Green-gray	19.0 19.4	4.0 4.0	.....	.....	.....
	3 months' storage at —20°F.	2.3	<100	Green-gray	16.5 16.5	4.5 4.0	8.2	2.0	7.1
	3 months' storage at 95°F.	2.4	<100	Lavender-gray	24.2	38.0 36.0	7.8	2.4	6.2

[1] Miss Boggs' method of scoring will be described elsewhere. In order to understand the data in this table, it should be stated that a score of 10 represents taste of good-quality fresh egg, and that numbers less than 10 represent tastes of decreasing desirability.
The scores reported here are not comparable with previous reports from this laboratory for the following reasons: (1) All samples were diluted with 50 per cent fresh egg before tasting; (2) all powders contained resazurin dye, the color of which may have had a psychological effect on the tasters; and (3) the uninoculated powders were prepared from aseptically collected egg liquid.

The plate counts of all of the inoculated powders were low or negligible at the end of the storage period. The free fatty acid content of the inoculated powders stored at 95°F. was increased 1,900 per cent over that of the corresponding uninoculated powders.[6] Since the values of the inoculated powders stored at —20°F. were approximately twice that of the uninoculated stored powder, some increase in free fatty acid content in the inoculated eggs before lyophilization may be indicated.

The colors of both inoculated and uninoculated powders (resulting from added dye) were green-gray before storage and therefore quite comparable. The color changes which occurred during storage confirm the findings of the previous experiment and are illustrated (Fig. 5). The inoculated powders increased the rate of dye-color change during storage above that shown by the uninoculated powders. The color changes noted in the stored uninoculated powders indicated the formation of dye-reducing substances by the egg components[7] during storage, and confirmed the findings reported in the companion communication that storage at 95°F. increased the rate of resazurin reduction by emulsions prepared from uninoculated powders.

The results of the previous experiment are also confirmed in relation to changes in salt-soluble and lipid fluorescence. The findings showed that the inoculated powders gave a higher salt-soluble fluorescence value before storage than the corresponding uninoculated powders, but did not give a higher lipid fluorescing value than the uninoculated powders either before storage or after 12 weeks' storage at —20°F. However, the lipid fluorescence value of the inoculated powder stored at 95°F. was greater than that of the uninoculated powder stored at the same temperature. The data lend support to evidence previously submitted from this laboratory indicating changes in the lipids to be factors in quality deterioration as shown by palatability scoring, Dutton and Edwards (1946) and Fevold, Edwards, Dimick, and Boggs (1946).

DISCUSSION

Throughout this study effort was made to keep growth of *Ps. fluorescens* in egg liquid at a minimum before lyophilization, so that the effects of growth products would not be confused with those formed subsequent to drying and during storage. No measurable growth (reproduction of bacteria) took place in the powders during storage—in fact, the viable counts

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[6] It seems desirable to point out that the Kline and Johnson (1946) method for estimating free fatty acid content excludes lactic acid as a factor in acidity.

[7] Olcott and Dutton (1945), Edwards and Dutton (1945), and Dutton and Edwards (1945) have shown that both salt-soluble and lipid fluorescence appear to be due to the presence of products resulting from Maillard reactions. Some of the products of this type of reaction have been demonstrated by Kline (1945) to possess strong reducing power. Model experiments with mixtures containing aldehydes (glucose and acetaldehyde) and amines (glycine and cephalin) were found to reduce resazurin when warmed. It is therefore possible that the reaction products of the egg powder components which appear to be responsible for fluorescence are also responsible for resazurin reduction, and the observed increases in fluorescence and dye reducing values of uninoculated powders during storage may be attributed to reactions between products which are present initially or formed during storage. Such products might be formed by enzymes, which are natural constituents of eggs or by nonenzymic reactions.

of the inoculated powders decreased so rapidly that the numbers of living bacteria were negligible at the end of 12 weeks' storage. Any products formed, therefore, in the inoculated powders during storage were due to the activity of nonreproducing cells, or to cell components (presumably enzymes) that remained active after the death of the organisms.
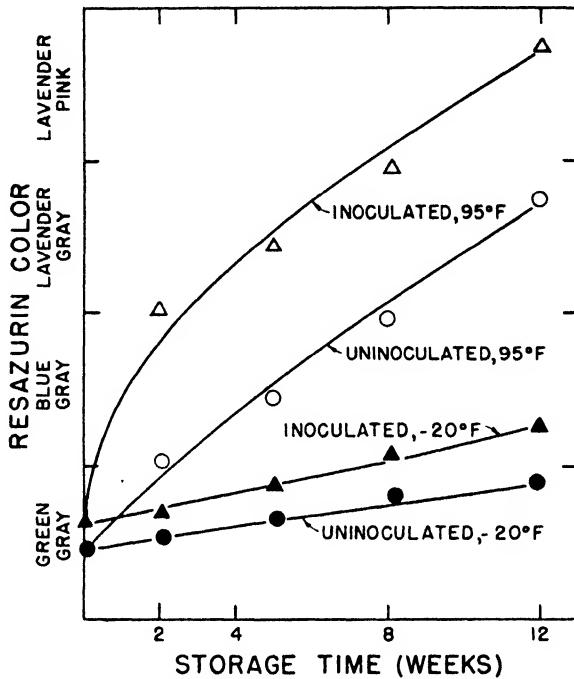
Fig. 5. Effect of *Pseudomonas fluorescens* on resazurin reduction[1] by lyophilized eggs.

[1] The dye was added to the emulsion before lyophilization; the color changes occurred in the "dry" powder.

As previously pointed out, the work reported here was limited to powders prepared by lyophilization. Although it cannot be argued that all microorganisms that might occur in egg liquids or commercial powders would produce similar changes, the results confirm the work of Stuart, Goresline, Smart, and Dawson (1945) on spray-dried powders collected from numerous drying plants and extend the findings by the use of quality tests other than solubility.

The effect of *Ps. fluorescens* on the values of the salt-soluble and lipid fluorescence tests seem noteworthy, since so far as is known, no previous report has been made on this subject despite the fact that salt-soluble fluorescence is widely used as a means of determining egg quality. Lipid fluorescence has been recently suggested for a similar purpose, Dutton and Edwards (1946).

These results show that undesirable changes attributable to bacterial activity may occur, even though the number of viable organisms as determined by plate count is negligible, and indicate a relationship between

microbiological history and storage life of the dried products. The studies emphasize, therefore, the importance of plant sanitation and, particularly, the necessity of using egg liquids of high sanitary quality.

SUMMARY AND CONCLUSIONS

Whole-egg liquids, uninoculated and inoculated with *Pseudomonas fluorescens,* were vacuum-dried from the frozen state. The resulting "low" moisture powders were stored for 12 weeks at 95 and —20°F. Salt-soluble and lipid fluorescence and number of viable bacteria were determined before and during storage. Similar egg powders to which resazurin dye solution was added before lyophilization were observed for dye-color changes during storage. After storage the powders were examined for free fatty acid content, palatability, pH, number of viable bacteria, and salt-soluble and lipid fluorescence. The findings were as follows:

(1) The additions of *Ps. fluorescens* caused increase in salt-soluble fluorescence before storage, the differences between initial values of the inoculated and the uninoculated powders being maintained during storage. (2) Incorporation of *Ps. fluorescens* did not increase lipid fluorescence values of the powders before storage or during storage at —20°F., but increased lipid fluorescence during storage at 95°F. over that of the corresponding uninoculated powders. (3) Inoculated powders, to which resazurin was added before lyophilization, increased the rate of dye-color change during storage above that shown by uninoculated powders; in general, the rate of resazurin reduction by inoculated and uninoculated powders increased with increasing temperatures. (4) Inoculated powders stored for 12 weeks at 95°F. showed marked increases in free fatty acid content, higher lipid fluorescence values, and increased resazurin color changes; inoculated powders stored at 95 and —20°F. showed lower pH and palatability values than corresponding uninoculated powders. (5) Over 99.9 per cent of the inoculated organisms failed to grow after drying, and the death rate of the surviving organims was very high during storage; the plate counts of all inoculated powders were negligible (<500 per gram) at the end of 12 weeks' storage.

It appears that deteriorative changes in inoculated stored egg powders, estimated by lipid fluorescence, free fatty acid content, pH, palatability, and resazurin color, are related to the activity of nonreproducing bacteria, or to cell components (presumably enzymes) that remain active after the death of the organisms.

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INFLUENCE OF FREEZING ON COLOR OF BONES
AND ADJACENT TISSUES

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The color of blood and muscle tissue is due to the presence of heme pigments. Hemoglobin gives blood its red color, whereas the red color of fresh uncooked muscle is dependent on the presence of myoglobin. Hemoglobin is a conjugated protein made up of an iron-containing portion, heme, and a protein-containing portion, globin. The myoglobin of the muscle is closely related to hemoglobin, although differences may be observed by means of the precipitin reaction according to Hektoen, Robscheit-Robbins, and Whipple (1928). Hemoglobin is contained within the red blood corpuscles or erythrocytes, which circulate in the blood and which originate principally in the red marrow of bones.

The blood and muscle pigments are not stable, but rather readily undergo reversible and irreversible changes. As might be expected, they are very susceptible to chemical reaction with oxygen, because their function is to supply oxygen to living tissues. Thus, arterial blood is relatively bright red owing to the combination of hemoglobin with oxygen (oxyhemoglobin), whereas venous blood is purple because of the presence of reduced hemoglobin.

Hemoglobin and myoglobin will oxidize in the presence of oxygen to form methemoglobin and metmyoglobin, respectively. Brooks (1929) observed in meat that metmyoglobin was formed most rapidly at some distance from the tissue-air interface. Ramsbottom and Koonz (1941) reported that the surface color of beef at the end of one year's storage was greatly influenced by the amount of metmyoglobin present in superficial lean tissue. More oxidation and darker beef occurred at —12.2°C. (10°F.) than occurred at —34.4°C. (—30°F.).

In unfrozen or frozen muscle metmyoglobin is formed very slowly. However, when frozen bones containing hemoglobin are defrosted they become dark in color very rapidly because of the accelerated development of methemoglobin. Immediately on defrosting, the bones may or may not be darker than they were in the frozen state. However, darkening proceeds rapidly in a defrosted bone, with maximum darkness being obtained between zero and five days after defrosting. Methemoglobin is formed in bones containing red marrow since this is a hemopoietic tissue and contains hemoglobin. Red marrow is found extensively in bones of younger animals and consequently more darkening will occur in the immature bones. In mature animals much of the red marrow is replaced by yellow marrow, which is not concerned with hemopoiesis and does not contain hemoglobin.

Hemoglobin and myoglobin are proteins capable of coagulation and denaturation by heat. Consequently, on cooking, the red color of fresh

meat changes readily to various shades of brown and grey because of the formation of hemochromogen and other breakdown products of myoglobin and hemoglobin. The bones from younger animals, such as broilers, are not completely calcified. The epiphyses of such bones characteristically have spongy walls and during freezing and defrosting some of the marrow fluid containing hemoglobin escapes through the porous walls of the bones. When such bones are cooked, their surfaces and even adjoining tissues may appear abnormally dark.

The purpose of the present study was to determine the extent of discoloration for defrosted bones, how it might be prevented, and whether darkening of tissues adjacent to bones in cooked poultry could be minimized.

EXPERIMENTS WITH BEEF, LAMB, AND VEAL BONES

An experiment was made to determine whether beef bones which were not cut before freezing would turn as dark as those cut prior to freezing. The 13th rib was used for this purpose. Rib bones were obtained from carcasses of both young and mature cattle. Some of the bones were split on a band saw before freezing at —34.4°C., others were split after defrosting. Some bones were held at 2.2°C.(36°F.) as controls, others were frozen once, and still others were frozen twice. It was found that the cut surface of the twice frozen and defrosted bones was darker than the cut surface of the bones frozen and defrosted only once. Bones which were frozen while intact were not as dark as those which were split before freezing. The cut surfaces of defrosted bones from young animals were much darker than those from mature animals.

The effect of quick-freezing on the color of beef bone was investigated. Thin sections of bone, one to five millimeters in thickness, were frozen in direct contact with dry ice and also indirectly, using pliofilm as a wrapper. The bones were thawed and held at 2.2°C. until inspected three days later. It was found that the frozen bones had darkened in color, whereas the unfrozen control samples remained a bright red.

In another experiment beef bones were frozen in an atmosphere relatively free of oxygen to determine if such a procedure would prevent darkening. This was done by replacing the air with nitrogen which had been passed over heated metallic copper to remove the small amount of oxygen normally present in commercial nitrogen. The results indicated that freezing and thawing in a nitrogen atmosphere did not prevent discoloration; apparently the small amount of oxygen present in the bone was sufficient to cause some oxidation. Similar results were obtained when sections of bone were sealed in "vacuumized" packages before freezing and thawing.

Veal and lamb carcasses were divided into right and left sides by splitting them down the vertebrae. Sections of the vertebrae were removed and frozen at —23.3°C.(—10°F.), and defrosted at 2.2°C. Adjoining sections were held at a temperature of 2.2°C. The unfrozen or control vertebrae remained bright red (Fig. 1), whereas the defrosted bones turned almost black. The photograph does not demonstrate the widespread difference in color that actually existed.

Other sections of unfrozen and defrosted veal vertebrae were crushed and water extracted in order to remove the pigment. The total hemoglobin pigment concentration was determined according to the method of Drabkin and Austin (1935) employing the data of Jensen and Urbain (1936) for oxyhemoglobin and Urbain and Greenwood (1940) for methemoglobin. Spectrophotometric analysis showed that 23 per cent of the hemoglobin present in the unfrozen veal bone was in the form of methemoglobin, whereas 62 per cent of the hemoglobin present in the defrosted bone was in the form of methemoglobin.

EXPERIMENTS WITH POULTRY

Comparisons were made between unfrozen and defrosted fryer-size chickens to determine the time required for bone darkening to take place. Immediately upon being defrosted, the bones from frozen poultry were
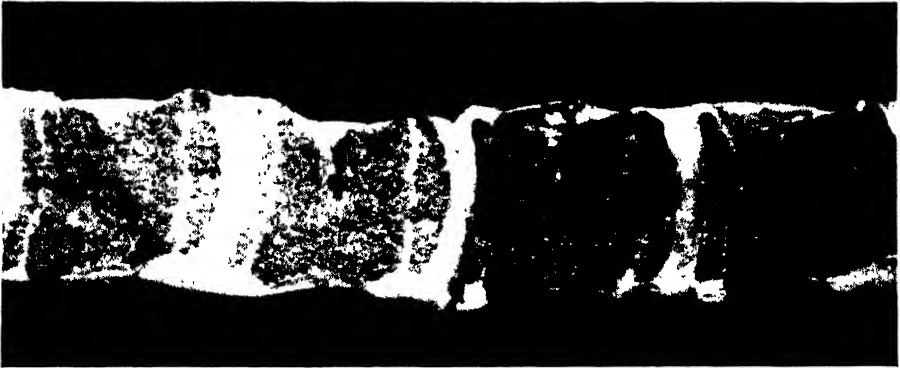
Fig. 1. Veal vertebrae, longitudinal sections.
Light-colored, unfrozen section.
Dark-colored, defrosted section.

bright red in color, similar to the unfrozen controls. However, upon storing, the defrosted bones became progressively darker, and after a period of several days the marrow turned almost black. Freezing apparently disturbed the cells containing hemoglobin. On exposure to air methemoglobin was formed at a rapid rate. Similar results were secured for flesh covered and defleshed bones. After the marrow was removed from the interior of long bones there was no difference in the appearance of shafts of defrosted and unfrozen bones.

The results of a number of tests designed to determine if changes in color of defrosted bones and darkening of tissues adjacent to bones could be prevented showed that the length of time that dressed poultry was held prior to freezing, the freezing temperature, or the method of dispatching had no influence on color (Table 1). The birds were cooked by roasting regardless of whether they were broilers, fryers, roasters, or fowl.

It was felt that some observations should be made on poultry of the various classes, cooked in the conventional manner for each class. An experiment was conducted in which two birds served as unfrozen controls,

TABLE 1

Influence of Method of Handling on Bone Darkening and Pigment Diffusion From Marrow to Adjacent Muscle Tissue

Factors tested		Class of bird	Number of birds	Days held prior to freezing	Freezing temperature (°F.)	Influence of factor on cooked appearance
General	Specific					
Method of dispatching	Decapitated	Fryer	6	1	-30	None
	Conventional	Fryer	6	1	-30	None
	Severed both jugulars	Fryer	6	1	-30	None
Special treatment during dispatching	Warm water-sprayed	Fryer	3	1	-30	None
	Mouth and throat washed with 2% sodium citrate	Fryer	5	1	-30	None
	In chamber having 20-inch vacuum	Fryer	3	1	-30	None
Time held prior to freezing and freezing temperature	Warm frozen [1]	Roaster	3	0	Brine -30	None
	Warm-frozen	Fowl	3	0	Brine -30	None
	Warm-frozen	Fryer	3	0	+8	None
	Warm-frozen	Fryer	3	0	0	None
	Warm-frozen	Fryer	3	0	-10	None
	Warm-frozen	Fryer	3	0	-32	None
	Chilled before freezing	Fryer	3	1	+8	None
	Chilled before freezing	Fryer	3	1	-10	None
	Chilled before freezing	Fryer	3	1	-32	None
	Chilled before freezing	Roaster	3	4	Brine -30	None
	Chilled before freezing	Fowl	3	4	Brine -30	None
	Chilled before freezing	Roaster	3	4	0	None
	Chilled before freezing	Fowl	3	4	0	None
Time held after defrosting	Cooking started without previous defrosting	Fryer	6	1	-30	Beneficial
	Rapidly defrosted	Fryer	3	1	-30	None
	Three days	Fryer	6	1	-30	None

[1] Placed directly in freezer without conventional chilling.

two were subjected to cooking without first being defrosted, and two were defrosted prior to cooking. This procedure was followed for the four classes. In comparing the cooked birds it was noted that when cooking was started without previous defrosting, broilers showed a very minimum of darkening of tissues adjacent to the bones. Also, there was much less darkening of bones in fowl than in younger broilers and fryers.

Further studies were made in which poultry was handled in five different ways as follows:

1. Controls—unfrozen;
2. Cooking started without previous defrosting;
3. Frozen immediately after being dressed (warm-frozen);
4. Frozen quickly at —28.9°C.(—20°F.) in wind tunnel;
5. Frozen slowly at —9.44°C.(15°F.) in still air.

Three classes of birds, namely fowl, roasters, and broilers, were used in connection with each of the five methods of handling. The broilers were 11 weeks old and the roasters were 18 weeks old when dressed. The fowl were average farm fowl. The poultry was cooked by roasting. In the cooked birds the color of leg, thigh, and breast bones was recorded. The color of the meat was evaluated in tissues adjacent to the thigh bone and the breast bone. Discoloration was evaluated as 5 none, 4 slight, 3 distinct, 2 marked, and 1 very marked; the results of this experiment have been summarized (Table 2).

The results revealed that broilers showed darker bones than the roasters, and the bones of roasters were darker than those of fowl. A similar trend was observed for the meat adjacent to the femur. This was to be expected since the epiphyses of long bones in the younger birds have a tendency to be less thoroughly calcified than in older birds. In well-calcified bones any pigment that may be contained in the marrow has difficulty escaping through the walls of the bone. Also, it is important to recognize that the long bones, such as the tibiotarsus and femur of mature birds, have more yellow marrow than those of younger birds. Yellow marrow is not concerned with hemopoiesis and therefore is lacking in red pigment.

The three classes of birds appeared to be somewhat similar with respect to the amount of discoloration shown in the white meat adjacent to the sternum. The sternum contained some pigment both in the young and more mature birds. During freezing, defrosting, and cooking the pigment may be released to the adjacent white meat which becomes somewhat discolored. Since the meat is white, any dislocation of pigment may be readily detected.

The darkening of bones or of tissues adjacent to bones as a result of freezing did not in any way affect the organoleptic properties of the product except for the effect it may have had on appearance.

SUMMARY

A series of experiments was made on the bones of beef, lamb, veal, and poultry to determine the color changes owing to freezing and to investigate procedures that might reduce or prevent the darkening of

TABLE 2

Influence of Freezing on Bone Darkening and Pigment Diffusion From Marrow to Adjacent Muscle Tissue

Method of handling	Class of bird	Number of birds	Bone darkening			Meat discoloration		Average score
			Leg	Thigh	Breast	Adjacent to breast bone	Adjacent to thigh bone	
Unfrozen..........	Broiler	15	4.1	3.4	4.3	4.2	3.7	3.9
Cooked-frozen [1]	Broiler	15	4.2	3.6	4.0	3.8	1.8	3.5
Warm-frozen [2]	Broiler	5	3.3	2.7	3.9	3.9	3.1	3.3
Fast-frozen..........	Broiler	15	3.2	2.3	4.1	3.5	2.1	3.0
Slow-frozen..........	Broiler	5	3.3	3.4	4.2	4.0	3.0	3.6
Unfrozen..........	Roaster	15	4.6	4.3	4.9	4.7	3.3	4.4
Cooked-frozen [1]	Roaster	5	4.4	3.9	5.0	3.7	3.7	4.1
Warm-frozen [2]	Roaster	5	4.0	3.0	3.6	3.3	3.8	3.5
Fast-frozen..........	Roaster	15	4.1	3.3	4.8	3.8	3.5	3.9
Slow-frozen..........	Roaster	5	4.2	3.9	5.0	4.0	3.6	4.1
Unfrozen..........	Fowl	2	5.0	5.0	5.0	4.1	5.0	4.8
Cooked-frozen [1]	Fowl	2	5.0	5.0	5.0	2.5	5.0	4.5
Warm-frozen [2]	Fowl	2	5.0	5.0	5.0	4.0	4.5	4.7
Fast-frozen..........	Fowl	2	5.0	5.0	5.0	3.7	5.0	4.7
Slow-frozen..........	Fowl	2	5.0	5.0	5.0	4.1	5.0	4.8

[1] Placed in the oven without previous defrosting. [2] Placed directly in the freezer without conventional chilling.

bones. Also, studies were made to determine if diffusion of pigment through the walls of incompletely calcified bones containing red marrow could be reduced or prevented. The results are summarized in the following statements:

1. When bones containing red marrow were frozen and defrosted, the hemoglobin was oxidized to methemoglobin and the color of the bones changed from red to shades of brown, grey, and black.

2. Some bones containing hemoglobin were dark as soon as thawed. In other bones, a few days were required for darkening to reach a maximum.

3. Darkening was promoted by splitting the bones before freezing, or by freezing the bones twice.

4. Bones of young animals generally contain more hemoglobin than those of more mature animals and consequently, when defrosted, turn darker.

5. In defrosted, immature chickens some of the pigment escaped through the porous, spongy, incompletely calcified walls of the bones and accumulated on adjacent tissues, which appeared darker when cooked. Pigment displacement from the breast bone to adjacent tissues occurred to about the same extent in the young and more mature birds.

6. Darkening of hemoglobin in beef and veal bones was not prevented by (1) fast freezing, even when the product was frozen in direct contact with dry ice at a temperature of —76.7°C.(—106°F.) ; (2) evacuating the air from the package; (3) replacing the air surrounding the bone with nitrogen.

7. The period of time between slaughter and freezing had no significant effect on the color of poultry bones or on the pigment displacement from the interior of bones to adjacent tissues.

8. Cooking minimized the color differences between defrosted and unfrozen bones.

9. The darkening of bones by methemoglobin formation or the darkening of tissues adjacent to bones through pigment diffusion does not in any way affect the organoleptic properties of the product, except for the influence it may have on appearance.

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SURVIVAL OF FOOD-POISONING STAPHYLOCOCCI ON NUT MEATS

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Although nut meats in the unbroken shell are considered to be sterile, the methods of shelling, handling, and storing and the chemical nature of the product lend themselves to gross contamination of the nut meats and prolonged survival of contaminants, Tanner (1944) and Wenzel and Black (1939). Furthermore, the practice of using nut meats in uncooked foods or of adding them to foods that have already been cooked suggests the possibility that nut meats may be of public-health significance as the source of food-borne pathogens.

In addition to the common air and soil bacteria that are usually present in large numbers on nut meats on the retail market several investigators have found coliform organisms, Clark and Booth (1940), Ostrolenk and Hunter (1939), Prucha (1936), Smith and Iba (1946), and Weinzirl (1929). Clark and Booth (1940) reported the presence of staphylococci on 24 per cent of 224 samples of nut meats. Geiger, Crowley, and Gray (1935) have isolated and identified *Staphylococcus aureus* as the cause of food poisoning from nut meats in ice cream. Bacteriological studies of the incriminated hazel nut, revealed excessively high bacterial counts (54 million per gram) of which *Staph. aureus* was the predominant organism (50 per cent).

In studying the survival of pathogens on nut meats Clark and Booth (1940) artificially contaminated meats with *Eberthella typhosa*, *Escherichia coli*, and *Staphylococcus aureus*. At room temperature the enterobacteria disappeared within five weeks, whereas the staphylococci survived for 10 weeks.

Because of their relative stability nut meats are stored at a wide variety of temperatures. Therefore, the experiments reported here were devised to study the influence of temperature on the survival of a food poisoning *Staph. aureus*.

EXPERIMENTAL PROCEDURE

Approximately 150 grams of small pieces of roasted pistachio nut meats were washed by shaking in tap water, followed by several washings in distilled water, and finally rinsed several times with sterile distilled water in a sterile flask. Aqueous suspension of 48-hour culture of *Staphylococcus aureus* grown on beef extract-peptone agar at 37°C.(98.6°F.) was poured into the flask containing the washed nuts and shaken thoroughly for a few minutes. The suspension liquid was poured out and counted by plate count. The contaminated nut meats were drained and then dried in a

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calcium chloride desiccator for about two and one-half hours until they appeared to be as dry as the original nuts.

One-gram quantities of the dried nut meats were weighed 'into sterile cotton-plugged tubes. Fifty tubes were stored in the refrigerator [4°C. (39.2°F.)], 40 tubes at room temperature [16 to 26°C.(60.8 to 78.8°F.)], and 40 tubes in the 37°C. incubator.

SURVIVORS' CURVE OF STAPHYLOCOCCUS AUREUS
ON
PISTACHIO NUTS

—— REFRIGERATOR
—*—*— ROOM TEMP
—•—•— 37°C.
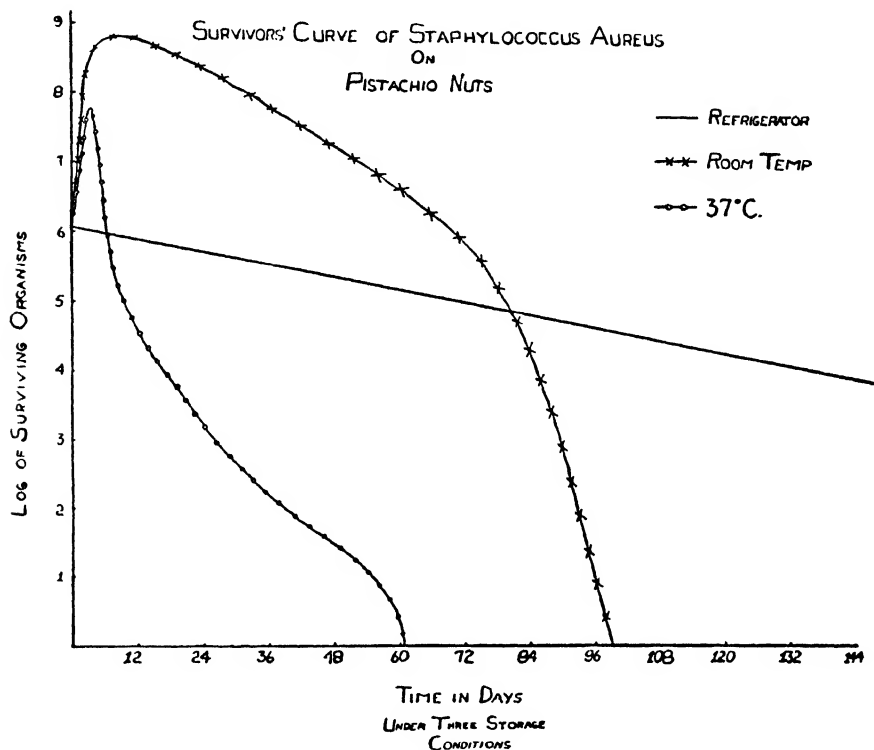
TIME IN DAYS
UNDER THREE STORAGE
CONDITIONS
FIG. 1.

One-gram quantities of nuts contaminated and stored in this manner were plate-counted immediately and at three-day intervals for 141 days or until successive counts showed no staphylococci. To perform a plate count the contents of a tube were shaken vigorously 25 times in a 100-c.c. water blank or, when the counts became low, in a 10-c.c. water blank. Undoubtedly only a portion of the organisms was removed, but this error was considered to be constant and was ignored. Appropriate sterile tap-water dilutions were plated in beef extract-peptone agar at pH 7.0. After 48-hour incubation at 37°C. and 24 hours at room temperature to improve pigment production typical colonies were counted on plates containing from 30 to 300 colonies.

The bacterial counts were recorded in the table and the shape of the survivors' curves appears on the graph in which the logarithms of the surviving bacteria are plotted against time. From these it will be noted that at room temperature and at 37°C. there was an initial increase in the

TABLE 1

Number of Viable Staphylococcus aureus Washed From Surface of One Gram of Broken Pieces of Pistachio After Various Periods of Storage

Number of days	4°C. (39.2°F.)	Room temp., 16-26°C. (60.8-78.8°F.)	37°C. (98.6°F.)
0	1,200,000[1]	1,200,000[1]	1,200,000
3	1,200,000	220,000,000	55,000,000
6	700,000	570,000,000	700,000
9	1,200,000	650,000,000	400,000
12	....	....	....
15	800,000	470,000,000	34,000
18	550,000	170,000,000	1,500
21	200,000	160,000,000	1,200
24	41,000	85,000,000	500
27	80,000	80,000,000	1,600
30	....	90,000,000	280
33	240,000	210,000,000	580
36	380,000	80,000,000	40
39	470,000	170,000,000	10
42	290,000	70,000,000	20
45	260,000	95,000,000	120
48	95,000	37,000,000	0
51	120,000	65,000,000	20
54	80,000	10,000,000	10
57	70,000	6,500,000	0
60	50,000	280,000	10
63	130,000	400,000	0
66	90,000	600,000	0
69	220,000	3,000,000	....
72	55,000	490,000	....
75	140,000	3,500,000	....
78	100,000	150,000	....
81	55,000	210,000	....
84	40,000	165,000	....
87	41,000	79,000	....
90	30,000	22,000	....
93	95,000	60	....
96	40,000	10	....
99	50,000	0	....
102	80,000	....	....
105	27,000	....	....
108	22,000	....	....
111	....	....	....
114	27,500	....	....
117	38,000	....	....
120	....	....	....
123	9,500	....	....
126	....	....	....
129	....	....	....
132	....	....	....
135	35,000	....	....
138	....	....	....
141	30,000	....	....

[1]The nuts were inoculated with suspension containing 28,000,000 organisms per cubic centimeter.

number of bacteria. This increase was followed by a decrease in viable staphylococci, the rate of decrease being related to the temperature of storage.

Viable *Staph. aureus* were present on refrigerator-stored pistachio nuts after 141 days, but after 99 days at room temperature or 63 days at 37°C. none could be detected.

These results were further substantiated by similar experiments with *Staph. aureus* on walnuts and hazel nuts.

<div align="center">DISCUSSION</div>

The straight-line curve of death at 4°C. follows the known rules of death of microorganisms. The unfavorable factors acted as constants and the relative resistance of the individual contaminants presents the only variable. The slight increase in resistance after 90 days, if it is real, is consistent with the prolongation of survival that is noted in the death phase of a bacterial-growth curve.

Similarly at 37°C. the death curve is readily explained. After an initial increase at the favorable temperature the combined antagonistic factors of dehydration and the metabolites acted as constants but, as above, the last ten thousand staphylococci showed a tendency to preserve the species and the rate of death was decreased.

The curve of death at room temperatures, however, is unusual. First the initial growth was much higher than the initial growth at 37°C. This may be an artifact that would not have appeared had daily counts instead of counts every third day been made, i.e., it is possible that at 37°C. a much higher count would have been detected on the first and second day but that by the third day enough organisms would have died to give an apparent lower maximum population.

Between the ninth and 84th day this room temperature curve reflects the death that would occur where constant factors act against a stable population of bacteria. At this point, however, instead of leveling off to prolong the survival of the culture, the rate of death increases very markedly to cause sterility after the 96th day. The cause of this increased death rate is unknown. It is possible that at that time a change in the atmospheric moisture, which would not be reflected in the cultures stored in the relatively drier atmospheres of the incubator and the refrigerator, took place and hastened the death of the organisms at room temperature. The possibility of metabolites from adaptive enzymes or from slow-growing antibiotic organisms should also be considered.

The individual variation between samples is not considered to be significant. The difficulty of preparing 130 tubes of contaminated nut-meat particles each with the same surface area and contamination was not resolved in these experiments.

The relatively rapid destruction of the pathogenic *Staph. aureus* at 37°C. has raised the question of the optimum temperature for storage of nuts. It is not believed that these data justify any change in storage practices. It is possible that if future experimentation showed other pathogens were also destroyed at 37°C. that temporary storage at this temperature might become part of a prestorage treatment. These experiments,

however, take no cognizance of the activity of spoilage organisms that might be more active at 37°C.

SUMMARY

Pistachio nut meats were experimentally contaminated with relatively large numbers of *Staphylococcus aureus* taken from agar cultures grown for 48 hours at 37°C. The nuts were divided into three lots, one lot being stored in a refrigerator (4°C.), one at room temperature (16 to 26°C.), and one at 37°C. At three-day intervals counts were made of the organisms surviving on one gram of nuts at each temperature.

Staph. aureus on nut meats at refrigerator temperature slowly die out but survive longer than 141 days.

At room temperature *Staph. aureus* increase in number reaching a maximum in nine days. The number of viable *Staph. aureus* then decreases until sterility occurs in 99 days.

At 37°C. the maximum count is reached within 48 hours after which there is rapid destruction. Viable *Staph. aureus* were not detected after 63 days.

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VEGETABLE CROPS IN RELATION TO SOIL FERTILITY

V. CALCIUM CONTENTS OF GREEN LEAFY VEGETABLES [1]

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Green leafy vegetables are recognized as important foods in the human diet. As providers of minerals and vitamins they are among the "protective" foods recommended by nutritionists. Attention has been called by Kohman (1939), Sherman (1944), and Wittwer (1945) to important nutritional differences between certain greens of the mustard family (kale, mustard greens, and turnip tops) and those of the goosefoot family (spinach, Swiss chard, beet greens, and New Zealand spinach). The superiority of greens of the mustard group may be ascribed to their contributions of calcium and ascorbic acid.

The importance of soil fertility as a determiner of "nutritive quality" in crops has been emphasized in a review by Beeson (1941). More recent reports of Holmes, Crowley, and Kuzmeski (1945); Lucas, Scarseth, and Sieling (1942); Sheets, McWhirter, *et al.* (1943); and Speirs, Anderson, *et al.* (1944) have continued to focus attention on this subject.

This report deals with comparative calcium values of some important green leafy vegetables, which were grown during the winter under controlled conditions in the greenhouse and with colloidal clay cultures.

EXPERIMENTAL PROCEDURE

The clay-culture technique of growing plants, using variable levels of calcium and nitrogen, was utilized. For a source of colloidal material the clay subsoil of Putnam silt loam was selected. This native material, leached of its exchangeable nutrients, has an exchange capacity of 28 milliequivalents per 100 grams, 12 of which are hydrogen. By replacing the adsorbed hydrogen with cationic nutrients and blending the clay with pure white, quartz sand, a clay-sand mixture results having the semblance of natural soil. The details of preparing the clay and adding the nutrients have been adequately described by Albrecht and Schroeder (1939).

In these studies a series of treatments was prepared by supplying calcium and nitrogen levels each of 5, 10, 20, and 40 milliequivalents (m.e.) with all possible (16) combinations of the two nutrients. Other ions were held constant for all treatments. The nutrient salt combinations and quantities of clay used to provide the 16 nutrient levels are presented (Table 1). The pH values of the cultures approximated 6.8.

The vegetables were grown in one-gallon glazed crocks with 10 replicates for each treatment. Seedling plants were allowed to develop for a

period of 60 to 90 days depending on the crop. At the proper stage of maturity the tops were harvested and the fresh and dry weights recorded. After being shredded in a Wiley mill and finely ground in a Merker mill, the dried material was suitable for analyses. Chemical determinations for calcium and magnesium were made according to the official A.O.A.C. (1940) methods. Oxalate was measured according to Pucher, Wakeman, and Vickery (1941).

TABLE 1

Amounts of Nutrient Salts and Clay Used in Providing Four Levels Each of Calcium and Nitrogen

Treatment No.	Variables (m.e.)		Salts used and milliequivalents of ions								Clay per plant (gm.)
	Ca	N	Ca–Ac		NH$_4$–NO$_3$		K$_2$H–PO$_4$		K–Ac	Mg–SO$_4$	
1	40	40	40	40	20	20	13.3 20		6.7 6.7	6 6	717
2	40	20	40	40	10	10	13.3 20		6.7 6.7	6 6	633
3	40	10	40	40	5	5	13.3 20		6.7 6.7	6 6	592
4	40	5	40	40	2.5	2.5	13.3 20		6.7 6.7	6 6	571
5	20	40	20	20	20	20	13.3 20		6.7 6.7	6 6	550
6	20	20	20	20	10	10	13.3 20		6.7 6.7	6 6	467
7	20	10	20	20	5	5	13.3 20		6.7 6.7	6 6	425
8	20	5	20	20	2.5	2.5	13.3 20		6.7 6.7	6 6	404
9	10	40	10	10	20	20	13.3 20		6.7 6.7	6 6	467
10	10	20	10	10	10	10	13.3 20		6.7 6.7	6 6	383
11	10	10	10	10	5	5	13.3 20		6.7 6.7	6 6	342
12	10	5	10	10	2.5	2.5	13.3 20		6.7 6.7	6 6	321
13	5	40	5	5	20	20	13.3 20		6.7 6.7	6 6	425
14	5	20	5	5	10	10	13.3 20		6.7 6.7	6 6	342
15	5	10	5	5	5	5	13.3 20		6.7 6.7	6 6	300
16	5	5	5	5	2.5	2.5	13.3 20		6.7 6.7	6 6	279

RESULTS AND DISCUSSION

The fresh weights of all the crops as influenced by the variable calcium and nitrogen are presented (Table 2). For all these vegetables the response to nitrogen was more marked than that for calcium. With New Zealand spinach and kale the calcium level in the cultures had practically no influence on the production of vegetation; whereas with spinach, chard, and mustard greens a pronounced interaction of calcium with nitrogen was noted. The amount of total vegetation produced varied with the crop and not with the botanical family to which it belonged. Yields of fresh material in spinach and beet greens were about half those for chard, turnip greens, New Zealand spinach, and kale. This would indicate that the latter collection of crops from the two families is not as exacting in the requirements for a high nutrient level in the soil as are spinach and beets, both of the goosefoot family. The yields, therefore, did not differentiate the two families.

The calcium contents of the crops, expressed in percentage compositions of dry weights, are assembled (Table 3) and portrayed graphically, as influenced by the calcium supplied in the clay cultures (Fig. 1). Marked differences in calcium values between greens of the mustard and goosefoot families are evident. In no case, regardless of the supply of exchangeable calcium in the soil, does the highest figure for the goosefoot greens (spinach

TABLE 2

Yields of Green Leafy Vegetables Grown at Variable Levels of Calcium and Nitrogen

Treatment No.	Variables (m.e.)		Fresh weight (gm. per 10 plants)						
	Ca	N	Spinach (Bloomsdale L. standing)	Swiss chard (Lucullus)	Beet greens (Detroit Dark Red)	New Zealand spinach	Mustard greens (Fla. Broadleaf)	Turnip greens (Shogoin)	Kale (Blue Scotch Curled)
1	40	40	234	635	264	468	341	429	523
2	40	20	171	486	154	422	270	342	308
3	40	10	85	233	72	268	179	187	163
4	40	5	37	180	46	142	77	94	66
5	20	40	321	487	276	280	268	411	544
6	20	20	220	694	160	368	274	338	370
7	20	10	109	243	95	231	177	193	146
8	20	5	65	134	48	123	103	112	71
9	10	40	179	392	226	461	235	356	468
10	10	20	229	334	132	325	279	307	351
11	10	10	139	215	90	219	182	165	192
12	10	5	115	117	66	122	99	92	90
13	5	40	129	32	192	466	254	310	558
14	5	20	205	213	186	392	284	269	401
15	5	10	153	172	124	159	212	178	223
16	5	5	60	105	69	99	97	83	88

TABLE 3

Calcium Contents of Green Leafy Vegetables Grown at Variable Levels of Calcium and Nitrogen

Treatment No.	Variables (m.e.) Ca	N	Contents of calcium on a dry-weight basis Spinach	Swiss chard	Beet greens	New Zealand spinach	Mustard greens	Turnip greens	Kale
			pct.	pct.	pct.	pct.	pct.	pct.	pct.
1	40	40	0.71	1.03	.76	.46	1.78	2.56	3.10
2	40	20	0.80	0.89	.52	.54	1.74	2.70	2.44
3	40	10	1.13	0.89	.92	.57	1.70	2.64	2.26
4	40	5	1.23	0.75	.86	.62	1.82	2.58	4.08
Average	...	...	0.97	0.89	.77	.55	1.76	2.62	2.97
5	20	40	0.69	0.78	.60	.66	1.64	2.42	2.46
6	20	20	0.64	0.68	.74	.48	1.60	1.91	2.24
7	20	10	0.75	0.72	.92	.55	1.38	2.32	1.75
8	20	5	0.86	0.78	.92	.63	1.54	2.56	3.20
Average	...	...	0.74	0.74	.80	.58	1.54	2.30	2.41
9	10	40	0.64	0.60	.86	.46	1.41	2.14	2.26
10	10	20	0.64	0.78	.56	.60	1.26	1.82	1.60
11	10	10	0.66	0.82	.66	.52	1.07	2.16	1.38
12	10	5	0.86	0.75	.76	.58	1.57	2.20	1.34
Average	...	...	0.70	0.74	.71	.54	1.33	2.08	1.65
13	5	40	0.79	0.49	.56	.55	1.50	2.22	1.98
14	5	20	0.59	0.82	.48	.53	1.25	2.00	1.48
15	5	10	0.68	0.66	.56	.51	0.99	2.26	1.38
16	5	5	0.62	0.55	.52	.51	1.24	2.04	1.66
Average	...	...	0.67	0.63	.53	.53	1.25	2.13	1.63

in this case) equal the lowest graphical value for any of the mustard group. Thus the amounts of dietary calcium supplied by the greens of the two families are widely different.
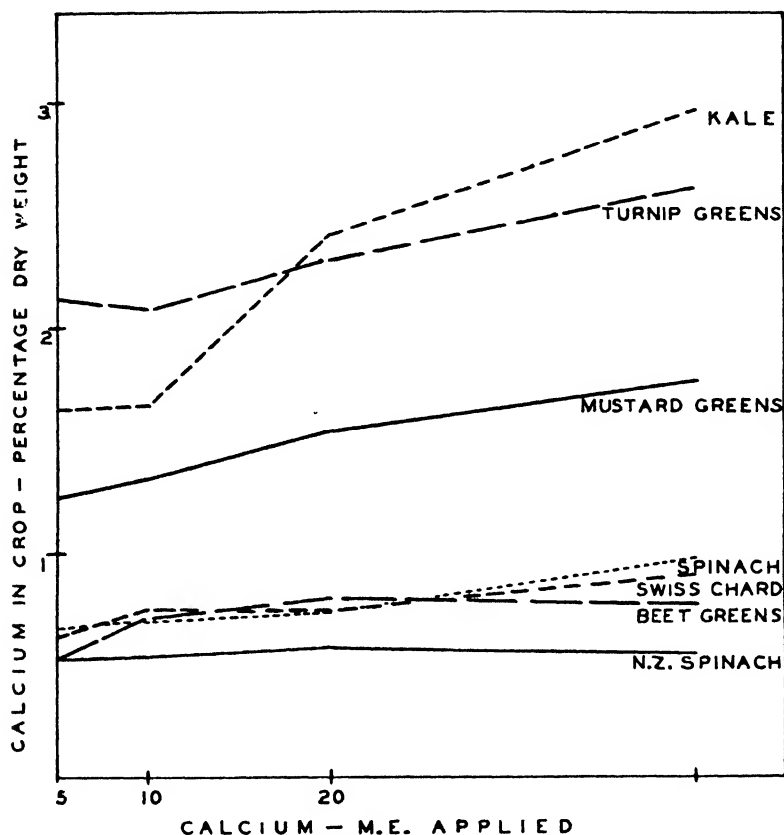
Fig. 1. Comparative calcium contents of green leafy vegetables.

As a result of increasing the calcium supply in the soil there were corresponding improvements in concentrations of calcium in the plant tissue. However, the increase in nutritional value in this respect was more pronounced in the mustard family. Of significance in this group was the betterment of quality, as concentrations of calcium in these vegetables, that was possible by additions of this nutrient element to the soil without any obvious external change in the appearance of the crop. Kale was one of the most responsive plants to an increased calcium supply, in so far as this altered its chemical composition, yet was influenced the least in its vegetative growth by the same nutrient. The calcium content was almost doubled without any apparent change in vegetative growth or appearance (Fig. 2).

These differences in calcium concentrations of the green leafy vegetables are greatly magnified when one considers differences in their nutritional

availability. According to Fairbanks and Mitchell (1938), Fincke and Sherman (1935), Kohman (1939), Speirs (1939), and Tisdall and Drake (1938) the calcium of spinach, Swiss chard, beet greens, and New Zealand spinach cannot be utilized in the diet because of the large amounts of oxalic acid present which combine with the plants' calcium and also with their magnesium to form insoluble and indigestible oxalates. In sharp
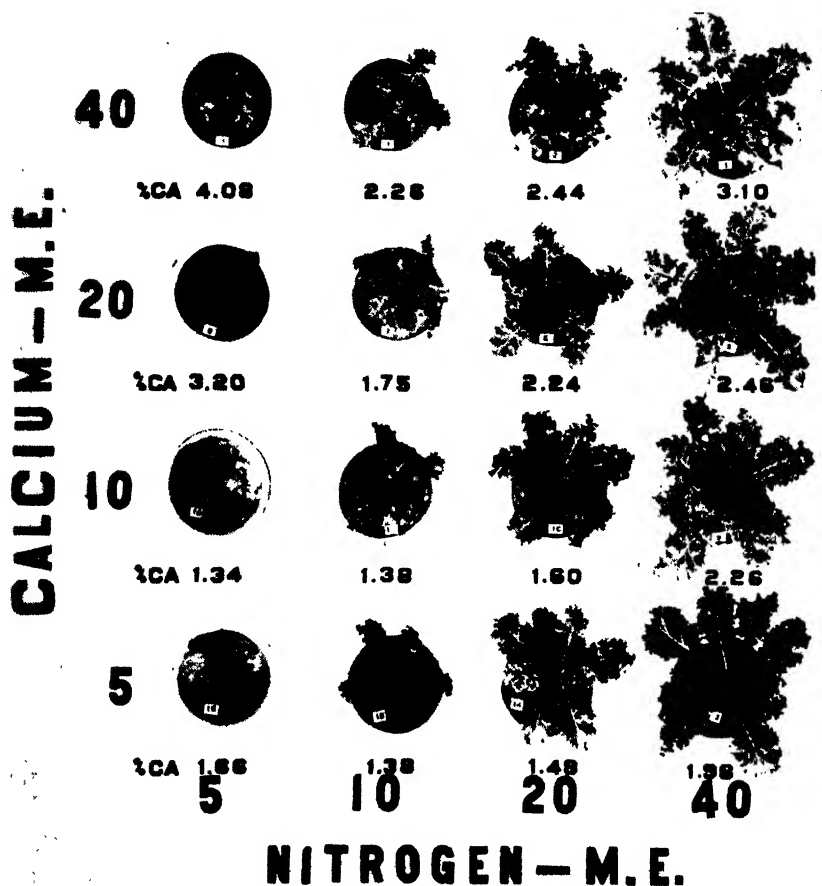
Fig. 2. Kale plants and their calcium contents when grown at variable levels of nitrogen and calcium. (Small numbers beneath each plant indicate the percentage in the crop on a dry-weight basis.)

contrast, according to the same investigators, the calcium of mustard greens, turnip tops, and kale is almost completely utilizable since these plants are practically free of oxalates.

With reference to spinach, Schroeder and Albrecht (1942) compared its nutritive quality when grown at variable levels of calcium in an acid soil (pH 5.2) and in another approaching neutrality (pH 6.8). The outstanding features of their experiments were the higher concentrations of

oxalate, calcium, and magnesium shown to be in the plants grown on the soil at a pH of 5.2. For this acid soil at all calcium levels the two bases, added together, were present in the crop in more than sufficient quantities to neutralize all the oxalic acid. The plants grown in near neutral soils (pII 6.8), however, failed to absorb sufficient calcium and magnesium for complete neutralization of their oxalate contents. Under the neutral conditions, increased calcium applications to the soil also failed to alter appreciably the concentration of calcium in the plant. In the experiments reported herein the clay cultures were prepared with pII values comparable to those approaching neutrality as used by Schroeder and Albrecht (1942). Therefore, high oxalate concentrations in relation to those of calcium and magnesium in the crops were anticipated.
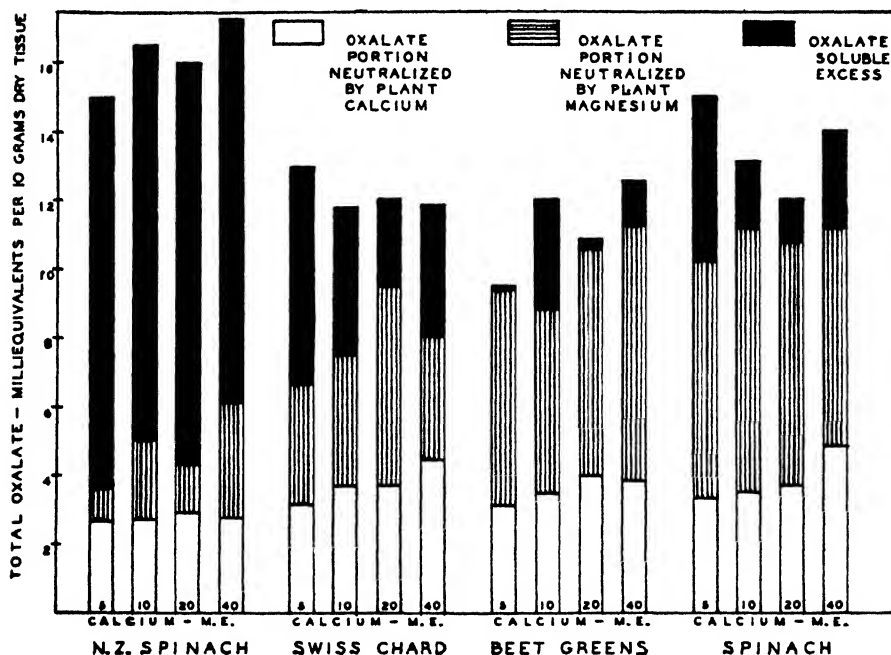
Fig. 3. Probable disposition of oxalate in New Zealand spinach, Swiss chard, beet greens, and spinach when grown at variable levels of calcium.

The total oxalates produced, including those portions neutralizable by the plants' calcium and magnesium as well as those in excess, are portrayed (Fig. 3) for the four crops of the goosefoot family. Expressed as milliequivalents per 10 grams of dry plant tissue, stoichiometrically the oxalate exceeded by several times the calcium at all fertility levels. Under the conditions of these experiments not one of the four crops contained sufficient calcium, or even enough calcium and magnesium combined, to neutralize all its oxalic acid. The condition of complete neutralization was most nearly approached in beet greens and spinach. According to the chart New Zealand spinach, Swiss chard, beet greens, and spinach could contribute no

dietary calcium. In addition, some excess oxalate was always present beyond the quantities possible of neutralization by the plants' bases. If one were to neutralize completely the oxalate of New Zealand spinach by calcium it would require from four to six times as much as the plant itself contains.

The effect on oxalate production of increasing the soil's calcium supply was not appreciable. It has been shown by Wittwer, Albrecht, and Goff (1946) that altering the level of soil nitrogen does, however, influence oxalate synthesis. It is of interest that the undesirable effects of excess, soluble oxalates in the goosefoot family were not overcome by the mere addition of more calcium to the clay media, and that in no case could all the oxalate be neutralized even when both the plants' magnesium and calcium were considered for that end.

<center>CONCLUSIONS</center>

The comparative calcium contents [2] of spinach, Swiss chard, beet greens, New Zealand spinach, mustard greens, turnip tops, and kale were ascertained by analyzing the crops, each grown under controlled greenhouse conditions in colloidal clay cultures.

Increasing the calcium supply in the substrate enhanced, in general, the calcium concentrations in the crops.

The members of the mustard family (turnip greens, kale, mustard greens) had a much higher percentage of calcium than those of the goosefoot family (spinach, Swiss chard, beet greens, and New Zealand spinach). The differences in calcium contributions to the human diet by the two plant families were magnified by the high oxalate content in the goosefoot greens. When this oxalate was expressed on a chemically equivalent basis, it was present in sufficient quantities to neutralize and thereby make insoluble and indigestible all the calcium and magnesium in these greens and to leave excess oxalate for dietary removal of calcium derived from other foods consumed with them.

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A SIMPLIFIED CHEMICAL METHOD FOR DETERMINATION OF NICOTINIC ACID IN PORK PRODUCTS [1]

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Several chemical methods have been published for the determination of nicotinic acid. Most all of these methods involve the reaction of nicotinic acid with cyanogen bromide and an amine. Teeri and Shimer (1944) have pointed out the difficulties frequently met with in these different methods and have proposed the use of m-phenylenediamine dihydrochloride with the addition of hydrochloric acid to stabilize the color.

In their method, distilled water is used for setting the instrument at 0 or 100 depending on the type of instrument used. The measurement of the reagent blank and the sample blank is required, and the sum of these two blanks is subtracted from the true nicotinic acid determination. The reagent blank is often greater than the sample blank, owing to the color in the amine reagent. This color increases when the amine reagent is allowed to stand in the laboratory.

Clarification of the amine reagent with charcoal and the use of commercially prepared cyanogen bromide for the preparation of the four-per cent cyanogen bromide solution gives practically a colorless reagent blank. The removal of color from the amine reagent with charcoal permits the addition of larger amounts of amine to be added to the tissue extract, which in turn has increased the sensitivity of the method approximately twofold. Increased sensitivity permits higher dilution of the extract and reduces the sample blank.

Since hydrochloric acid is used for stabilization of the developed color, it was found that by the addition of hydrochloric acid to the sample before the addition of cyanogen bromide and the amine no color was developed from the nicotinic acid present. It was, therefore, possible to combine the reagent blank and the sample blank into one solution and use this solution for setting the galvanometer. This procedure eliminates the readings of the sample blank and the reagent blank. It was felt that these improvements were worth publishing at this time, because they avoid certain difficulties met with in the determination of nicotinic acid by chemical methods. The chemical method described is less time-consuming than the microbiological method.

These results were obtained from an Agricultural Experiment Station Project designed for studying the influence of ration and storage in freezer lockers on the nicotinic acid potency of pork and they are presented here

to show that a close agreement can be obtained between the microbiological methods and the proposed chemical method on pork products.

Acid-Alcohol-Charcoal: Dilute 95 per cent alcohol to 80 per cent with 6 N hydrochloric acid. Add 10 mg. of charcoal per milliliter (Darco Vegetable Charcoal obtained from the Coleman & Bell Company, Norwood, Ohio) of acid alcohol. Shake or stir vigorously.

Cyanogen Bromide: Prepare a four-per cent solution by dissolving commercially prepared cyanogen bromide Eastman No. 919 in distilled water.

Amine Reagent: Dissolve 50 grams of m-phenylenediamine dihydrochloride Eastman No. 206 in 500 ml. of distilled water in a glass-stoppered bottle. Add 10 to 12 grams of Darco animal charcoal and stir for one hour with a mechanical stirrer. Allow charcoal to settle and filter the amount needed. Unused excess filtrate is returned to the original bottle. Very little or no color develops over a period of time in the presence of charcoal. If a slight amount of color develops, add 1 to 2 grams more of charcoal to the amine reagent.

Buffer Solution: Add to 1,850 ml. of water, 10 ml. of 85 per cent phosphoric acid, 50 ml. of 15 per cent sodium hydroxide, and 250 ml. of 95 per cent alcohol. Adjust to pH 6.6.

Hydrolysis of Sample and Preparation of Extract: Transfer five grams of animal tissue to a large test tube that has been previously calibrated to make up to a volume of 50 ml. Add 30 ml. of 6 N hydrochloric acid and hydrolyze for one hour with occasional stirring in a boiling-water bath. Remove tubes from water bath, cool, and make up to volume of 50 ml. with water. Mix contents well in the tube and filter through Whatman No. 31 filter paper. Transfer 15 ml. of the filtrate to an Erlenmeyer flask containing 15 ml. of the freshly prepared acid-alcohol-charcoal. Stopper the flask and shake for 30 seconds; then filter through a Whatman No. 2 filter paper, pouring back into the funnel the first 10 ml. of the filtrate. Transfer 20 ml. of the filtrate to a 50-ml. volumetric flask. Add 5 N sodium hydroxide until alkaline to phenolphthalein, add distilled water to make approximately 40 ml., and then make neutral to litmus with 20 per cent phosphoric acid. Dilute contents to 50-ml. volume with distilled water.

Determination of Nicotinic Acid: Transfer five ml. of the above tissue extract to each of two 50-ml. Erlenmeyer flasks. To one flask add three ml. of 3 N hydrochloric acid. To each flask, add five ml. of buffer reagent and five ml. of four per cent cyanogen bromide. Allow solutions to set for 20 minutes. To the flask containing the three ml. of 3 N hydrochloric acid, add two ml. of the amine reagent from a two-ml. pipette. Mix well and transfer to a colorimetric tube from an Evelyn photoelectric colorimeter. Place tube in the colorimeter and set galvanometer to read 100, using a 420-millimicron filter.

To the other flask add two ml. of the amine reagent with a pipette. Mix thoroughly and allow 30 to 40 seconds for maximum color development and then add immediately three ml. of 3 N hydrochloric acid from a burette with constant shaking of the flask. Transfer this solution to a colorimetric tube and read galvanometer within five minutes from the time of addition of the amine.

The addition of hydrochloric acid after addition of the amine at the proper time is one of the critical steps in the procedure. Allowing approximately five seconds for the delivery of the amine from the pipette, 30 to 40 seconds should elapse after the addition of the amine before the addition of hydrochloric acid. Consistent results were obtained by this procedure.

Calibration of the Photoelectric Colorimeter: Five ml. each of solutions containing 0.5 to 5.0 micrograms of nicotinic acid per milliliter were used for calibration of the constant for the instrument. $K_1 = \dfrac{L}{C} = .0216 \pm .004$. C is the number of micrograms of nicotinic acid present, and L is the log value of the galvanometer reading taken from the log tables supplied with the Evelyn photoelectric colorimeter. Values of K were found to be constant over the working range of the galvanometer. It is recommended, however, that a constant be determined for each series of determinations especially when new reagents are prepared. The values given for K above are values obtained in this laboratory under the conditions described and are given here only for comparison of sensitivity with other methods.

When a five-gram sample is used and the same dilutions are made as described in procedure, the equation for calculation of results is milligrams of nicotinic acid per 100 grams $= K_2L$ where $K_2 = \dfrac{10}{K_1}$.

RESULTS AND DISCUSSION

The procedure described for obtaining the tissue extract for the determination of nicotinic acid is essentially that of Melnick and Field (1940a, b). Larger samples were taken for hydrolysis insuring a more representative sampling, and instead of weighing the charcoal for each sample the charcoal is added to the acid alcohol and an aliquot of this mixture is used for clarification of the extract.

To determine the percentage of recovery, nicotinic acid was added to the extracts; the percentage recovery is shown (Table 1). Duplicate analyses of liver tissue made chemically and microbiologically are shown in the last two columns of the same table. These results show that a good recovery is obtained by the chemical method, and the variation between duplicate samples and the microbiological and chemical method is small.

Typical results for meats obtained by microbiological method of Krehl, Strong, and Elvehjem (1943) and the proposed chemical method are shown (Table 2). These different cuts and samples of meats were taken from pigs on rations with different nicotinic acid content, which accounts for the difference in nicotinic acid content of the different samples. Chemical analyses show slightly higher results than the microbiological assay. In spite of this difference, a good agreement was obtained for the two methods.

TABLE 1
Percentage Recovery of Nicotinic Acid and Comparative Values Between Chemical and
Microbiological Methods of Liver Extracts

Sample No.	Nicotinic acid in extract	Nicotinic acid added	Total nicotinic acid found	Recovery	Nicotinic acid content	
					Chemical	Microbiological
	μg.			pct.	mg./ 100 gm.	mg./ 100 gm.
1	10.4	5	15.6	104	16.6	17.5
	10.6	5	15.8	104	17.0	16.5
2	11.4	5	16.5	102	18.2	17.5
	11.2	5	16.0	96	17.9	17.5
3	8.67	5	13.8	102	13.9	13.0
	9.12	5	14.2	100	14.6	13.5
4	13.3	5	18.4	102	21.3	21.5
	13.3	5	18.2	98	21.3	21.5
5	11.3	5	16.2	98	18.1	18.2
	11.3	5	16.1	96	18.1	18.2

The variation in nicotinic acid content of the different samples of meat is indicated by both methods.

The addition of hydrochloric acid decreases the sensitivity of the method; this factor is negligible in comparison with advantages gained from its addition. Hydrochloric acid not only stabilizes the color after it has been developed for a period of time, but its use in the blank eliminates the reading of the reagent blank and sample blank. Also, the solution used for the reference reading has the same density as the sample used for the determination of nicotinic acid. In the presence of hydrochloric acid no color is developed when 500 micrograms of nicotinic acid are added, which is approximately 100 times the amount needed for a nicotinic acid determination.

TABLE 2
Typical Results of Chemical and Microbiological Methods in Determination of
Nicotinic Acid in Pork Meats

Description	Number of samples analyzed	Loins [1]		Hams [1]		Livers [1]	
		Chem.	Micro.	Chem.	Micro.	Chem.	Micro.
Lot 1-44........................	10	2.76	2.71	3.21	2.99	......	......
Lot 1-45........................	10	7.95	7.65	8.41	7.99	18.02	18.20
Lot 2-45........................	10	9.54	9.15	9.15	8.97	18.80	18.20
Lot 3-45........................	10	9.13	8.66	8.78	8.28	17.78	17.29
Lot 4-45........................	10	9.99	9.33	9.02	8.42	15.64	14.65
Lot 5-45........................	10	9.21	9.60	9.49	9.16	17.97	18.45
Total av........................	....	8.10	7.85	8.01	7.64	17.64	17.36
Per cent differences [2].....	....	+3.18	......	+4.62	......	+1.61	......

[1] Milligrams of nicotinic acid per 100 grams of meat.   [2] Microbiological method used as the basis of 100.

Preliminary work in this laboratory has shown that an increase in color is obtained in the presence of hydrochloric acid when the amine is added to a plant extract clarified with charcoal. This increase in color was not observed when the amine was added to the meat extract under the same conditions. These results would indicate that the method is more specific than previous methods for the determination of nicotinic acid in plant tissue. The presence of other substances which react with the amine in plant extracts has been reported by Kodicek (1940) and Waisman and Elvehjem (1941), and various methods have been proposed by Brown, Thomas, and Bina (1942, 1943) and Hausman, Rosner, and Cannon (1943) for the removal of these interfering pigments from plant-tissue extracts.

Investigations are under way to determine whether comparable results with the microbiological method can be obtained by this method with extracts of plant tissue.

SUMMARY

A rapid simplified procedure is described for the determination of nicotinic acid in pork meats. The tissue is hydrolyzed with 6 N HCl; the extract is clarified with charcoal, neutralized, and an aliquot taken for nicotinic acid analysis. Only one setting of the galvanometer is required for a single determination of nicotinic acid. The feasibleness of the use of one galvanometer setting has been made possible by clarification of the amine with charcoal, the use of commercially prepared cyanogen bromide, and the addition of hydrochloric acid to the sample blank before the addition of the four-per cent cyanogen-bromide solution and the amine. It has been found that the adding of these reagents to a solution strongly acidified by hydrochloric acid develops no color from the nicotinic acid present.

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DRIP AS A CONSTANT FOR QUALITY CONTROL OF FROZEN FOODS

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No satisfactory method exists at present for quality control of frozen foods. Several methods have been proposed and some of them have been extensively used in studies concerning the methods of freezing and the effects of various processes upon the product. Among these methods for quality control the most important are the test for vitamin C, the peroxide value of the fat, and the count of microorganisms, Tressler (1945). The indole reaction of oysters, Beacham (1946), and the electrometric titration of haddock, Stansby and Lemon (1933), have not as yet been well established as methods for quality control. The value of some other methods proposed (histamine determination, Geiger (1944), pH value determination) is still questioned. The test for tenderness, DuBois and Lee (1941), and the alcohol-insoluble solids test, Lee (1943), which may be useful in certain cases, have very limited application.

Of the first three methods—the vitamin C test, the peroxide value, and the count of microorganisms—none has general application. All three are complicated and their application in industrial work would be rather difficult. There is, furthermore, a marked disadvantage in that a record of the prefreezing condition of the product is needed for the interpretation of the results obtained by them. A mere statement of the number of microorganisms present would in many cases be of limited value without further information about the kind of microorganisms present. There is another property, however, which could be used satisfactorily in almost all cases of quality control of frozen foods. This property, known as the drip, practically corresponds to the result of the changes occurring during freezing, and its interpretation does not necessarily require any record of the prefreezing condition of the product as is the case with the above-mentioned methods. Drip, as used in this paper, denotes the liquid which exudes from the frozen product during thawing. Because no adequate measure of drip exists at present, very few values of drip or subsequent interpretation are found in the current literature. As a matter of fact values for drip appear in only a few papers. Five of these papers concern meat—DuBois, Tressler, and Fenton (1940), Ozoleng and Piotov (1941), Suarez (1928), and Ramsbottom and Koonz (1939, 1940)—and two concern fruits and vegetables—Joslyn and Marsh (1933) and Fieger, Dubois, and Kaloyereas (1946). Moran (1931), Heiss (1936), and Ray (1938) give only general information in their papers and not definite figures on drip. Even in those cases where definite figures for drip are given their value is questioned since there is such large variation in the results that

no conclusion can be based upon them—Ozoleng and Piotov (1941) and Joslyn and Marsh (1933).

In experiments on freezing fruits and vegetables by various methods, especially cauliflower, spinach, mushrooms, and strawberries—Kaloyereas (1940) and Fieger, DuBois, and Kaloyereas (1946)—determinations of drip were made, but little use was made of the data obtained because of the great variation between similar samples. The cause of these variations is the inadequate method used in determining the drip. The method used consisted of allowing the product to thaw and then to drain for two minutes over a mesh screen.

A new method for determining drip was developed in this laboratory using petroleum ether or Skelly Solve B previously saturated with water. The frozen sample is put into a beaker or other suitable container, covered with petroleum ether, and allowed to thaw for 24 hours. Then the sample is drained over a mesh screen and the volume of aqueous phase is read directly in a graduated cylinder. This method of determining drip always gives comparable results. Drip curves for various products, the drip being determined by both the old and the new methods, are shown (Fig. 1). The form of the curves obtained by the old method shows that there is no practical end to the exudation of drip even if the product is allowed to stand for more than 24 hours. On the other hand, by the new method a state is reached (after 4, 12, or 20 hours, according to the product) when practically no more drip is obtained from the product. When this condition of equilibrium is reached, the volume of drip is practically the same for similar samples frozen under identical conditions and can be taken as a constant for that particular product under the specified conditions of the experiment. The reason that stable values for drip are not obtained by the old method is that deterioration and fermentation changes take place. These changes, together with the unequal mechanical forces acting upon the various surfaces of the product during thawing, always create an unstable condition which never reaches equilibrium. Even if such equilibrium could be reached, it would not represent a normal condition of the product. This is shown by the rather large changes in pH and refractive index of the drip during the time of thawing (Table 1).

When spinach was tested by the old method, the pH and refractive index of the drip after three hours of thawing at room temperature were 6.15 and 2.8, respectively, and at the end of 24 hours were, correspondingly, 7.90 and 3.3. On the other hand, by the new method of obtaining drip, the pH and refractive index remained constant during the whole process of collecting the drip with value of 6.60 for pH and 2.8 for refractive index. With tomatoes similar results were obtained. The drip by the old method started with an initial pH value of 3.15 and increased to 4.20, while the refractometer reading changed from 5.5 to 4.7. Using the new method the pH changed from 3.40 to 4.00 and the refractometer reading from 5.6 to 5.8. In the case of meat the changes were still greater. The beginning values for pH and refractometer were 5.25 and 15.0, respectively, while at the end these same properties were 5.70 and 7.7, respectively. With the ether method the drip of the same meat had corresponding values for pH and refractometer of 5.50 and 13.5 in the begin-

TABLE 1

Comparison of Drip Obtained by the Two Methods

Product and method		After 3 hours			After 6 hours			After 18 hours			After 24 hours		
		Drip	pH	Refractive index (scale reading)	Drip	pH	Refractive index (scale reading)	Drip	pH	Refractive index (scale reading)	Drip	pH	Refractive index (scale reading)
		ml./100 gm.			ml./100 gm.			ml./100 gm.			ml./100 gm.		
Beef	Old method	1.0	5.25	15.0	1.7	5.50	13.0	3.3	......	......	4.2	5.70	7.7
	New method	1.1	5.30	14.5	1.6	5.30	14.5	2.2	5.40	13.8	2.2	5.40	13.8
Spinach	Old method	10.0	6.15	2.8	18.4	6.03	3.2	24.5	......	......	26.5	7.90	3.3
	New method	24.0	6.60	2.8	24.0	6.60	2.8	24.0	6.60	2.8	24.0	6.60	2.8
Cauliflower	Old method	1.7	......	6.6	2.7	......	5.6	5.0	......	......	6.0	......	6.6
	New method	8.1	6.20	4.7	8.6	6.20	4.7	9.5	6.20	4.7	9.5	6.20	4.7
Strawberries	Old method	23.0	3.55	7.8	41.0	3.33	7.0	50.0	......	......	58.0	3.02	6.0
	New method	20.0	3.30	6.6	24.0	3.25	7.0	2.90	3.25	7.0	30.0	3.25	7.0
Tomatoes (sliced)	Old method	17.6	3.15	5.5	37.6	4.20	5.0	51.0	......	......	58.5	4.20	4.7
	New method	35.7	3.40	5.6	44.3	3.50	5.7	50.0	4.00	5.8	53.0	4.00	5.8

ning and of 5.40 and 13.8 at the end. Changes in pH of meat have a definite effect upon the drip, according to Sair and Cook (1938). On the other hand, when the product is covered with petroleum ether, further changes are practically minimized until the condition of equilibrium is reached. In this case the mechanical pressure upon the different parts of
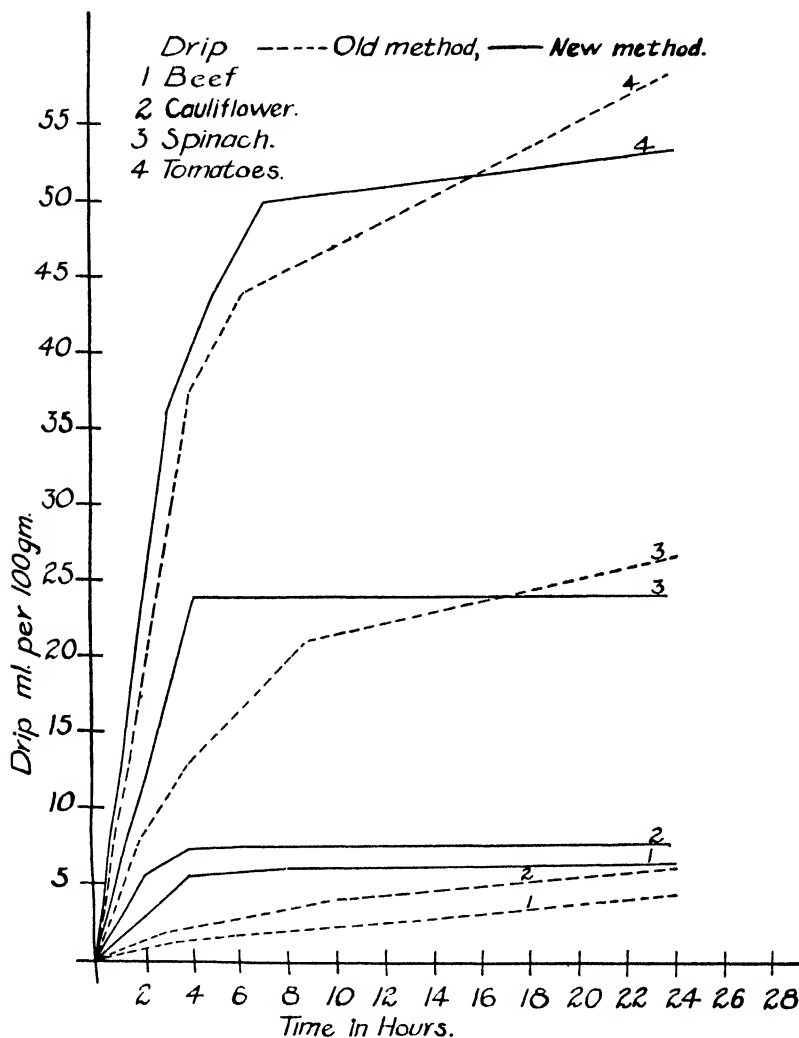
FIG. 1

the product during thawing is practically the same. The fact is confirmed by the constant values obtained for pH and refractive index in the drip. Determination of drip by the new method on various products frozen dry in still air at —17.8°C.(0°F.) and stored for one or two days at the same temperature, gave the data (Table 2).

The method can be applied also to fruit frozen with sugar (dry sugar or syrup) as well as with vegetables packed with brine. Treatment of

syrups with petroleum ether showed no change in refractive index of the syrups. In order to determine the extent of the practical application of this new method and its limitations for routine work, peaches of different varieties were packaged with 50 per cent sugar syrup in the proportion of three parts peaches and one part syrup by weight. The varieties used, whose freezing properties are well established, were Early Elberta, an excellent freezer; Georgia Belle, which is considered a poor freezer; and Elberta, which comes between these two. The freezing was done at 0°F. in air, and on freezer plates at—34.4°C.(—30°F.). Drip was determined

TABLE 2

Drip of Various Products Using New Method

Frozen product	Drip	pH	Refractometer reading
	ml./ 100 gm.		
Tomatoes, slices	53.0	4.00	5.8
Celery, stalks	25.0	......	3.2
Spinach, leaves	24.0	6.60	2.8
Lettuce, leaves	24.0	5.15	2.7
Pumpkin, segments	16.0	6.00	3.0
Potatoes, whole	10.0	6.00	5.0
Cauliflower, segments	9.5	5.98	4.7
Eggplant, slices	8.0	4.30	3.7
Okra, pods	1.0	4.20	5.0
Strawberries, whole	30.0	3.36	7.8
Peach, slices	16.5	3.65	8.3
Sultanina grapes	12.5	3.20	16.4
Cantaloupe, segments	12.0	5.92	8.2
Sand pear, slices	7.0	3.63	10.0
Avocado, slices	3.0	5.15	7.0
Beef, segments	2.2	5.40	13.8
Shrimp, tails	3.4	7.65	6.9

also on strawberries (Klonmore variety) packed dry, packed with dry sugar, and with syrup and frozen in air at 0°F., and by immersion in alcohol-dry ice bath at —65°C.(—85°F.). The results obtained are given, after different periods of storage (Figs. 2 and 3). It will be noted (Fig. 2) that the amount of drip is in inverse relationship to the freezing suitability of the different varieties. The drip, therefore, taken by this new method may serve as an index of the freezing properties of the different varieties of peaches. The values for drip for each product should always be examined comparatively and not be considered as absolute values since various factors, as shown later, affect drip.

A study of Fig. 3 brings out the effect of quick-freezing, the effect of time of storage, and the effect of the use of syrup and sugar on the volume of drip. In general, more rapid freezing causes decreased formation of drip. Storage, on the other hand, increases it.

The effect of quick-freezing upon the drip with various products is shown graphically (Fig. 4) where drip curves are drawn for five different products frozen in still air at 0°F. and by immersion in alcohol-dry ice

Fig. 2 (left chart)

Drip ml per 100 gm.

Time in Hours.

Drip of peaches.
1 Early Elberta packed in syrup.
2 Elberta packed in syrup.
3 Georgia Belle packed in syrup.
— Frozen in air 0°F.
--- Frozen on freezer plate -30°F.
A Packed dry frozen by immersion -8.5°F.
B Packed dry frozen in air 0°F.
C Packed dry frozen in air 0°F old method.

Fᴵɢ. 2

Fig. 3 (right chart)

Drip ml per 100 gm.

Time in Hours.

— Drip by New Method.
--- Drip by Old Method.
1 Strawberries frozen by immersion -8.5°F packed dry.
2 Strawberries frozen in air 0°F packed dry.
3 Strawberries frozen in air 0°F packed dry stored 4 ma
4 Strawberries frozen in air 0°F packed with sugar.
5 Strawberries frozen in air 0°F packed with syrup.

Fᴵɢ. 3

Frozen by Immersion -85°F
---- Frozen in Air 0°F
1 Beet
2 Peaches
3 Spinach
4 Tomatoes
5 Strawberries.

Drip ml per 100gm.

Time in Hours.

Fig. 4

bath at —85°F. In all cases where a considerable distance between the corresponding horizontal parts of the curves exists there is always a positive effect of quick-freezing upon the quality of the frozen pack. It must be realized, however, that the effect of quick-freezing upon the drip is not always the same with different products, and even quick-freezing does not always reduce the drip as is shown with Sultanina grapes, sand pears, and potatoes (Table 3).

This point is quite important since there is a rather general belief among various workers that the most rapid freezing always gives the best product. It has been observed that rapid freezing renders the product

firmer after thawing and is very desirable for most of the products frozen at present. There are, however, some products, such as Sultanina grapes, for which very rapid dry-freezing offers no advantage, especially if it is applied by the use of very low temperatures. There appears to be a defi-

TABLE 3

Effect of Rate of Freezing Upon Quantity of Drip

Frozen product	Drip from product	
	Frozen in air at —17.8°C. (0°F.)	Frozen by immersion in alcohol-dry ice bath at —65°C. (—85°F.)
	ml./ 100 gm.	*ml./ 100 gm.*
Tomatoes	53.5	19.5
Spinach	24.0	13.0
Eggplant	8.0	4.0
Pumpkin	16.0	14.5
Cauliflower	9.5	7.8
Okra	1.0	1.0
Strawberries	30.0	19.0
Peach	16.5	7.0
Avocado	3.0	1.0
Cantaloupe	12.0	8.0
Beef (after storage)	6.4*	4.7
Potatoes	10.0	14.0
Sultanina grapes	12.5	13.5
Sand pears	7.0	9.5
Sweet potatoes	2.0	1.0

nite relationship between the amount of drip and the bound water of the tissues. Since for products of high bound-water content low freezing temperatures affect the bound water-free water equilibrium to a greater extent than do high freezing temperatures, quick-freezing would not be

TABLE 4

Bound Water and Drip From Longfellow Pumpkins After Various Treatments

Treatment of sample	Bound water [1]	Drip
	pct.	*ml./ 100 gm.*
Untreated	2.46	16.0
Soaked in 55% sugar solution for 24 hours	8.64	0.5
Soaked in 1% pectin solution for 24 hours	4.40	10.8
Soaked in 2% gelatin solution for 24 hours	3.00	14.5
Dried partially in a vacuum desiccator	5.72	1.5

[1] The drying method of Nelson and Hulet for the determination of bound water was used in these experiments.

as beneficial for such products as for those of low bound-water content. Further work on this phase of the problem is being undertaken in this laboratory. By treating pumpkins, a product of low bound-water content, with substances such as sugar, pectin, gelatin, etc., which might affect the

capacity of tissues for binding water, results were obtained which seem to warrant the conclusion that there exists such a relationship between bound water and drip (Table 4). These results also throw new light upon the heretofore unexplained effect of sugar and pectin on fruit and vegetables during preservation by freezing. When Kaloyereas (1940) published results upon the use of pectin in freezing fruits and vegetables, the idea was then expressed that pectin acts in reducing drip by its gelling power. A better explanation lies in the effect of pectin on the bound water of the tissues. The inverse relation which always exists between the amount of drip and its refractive index suggests that fluctuation of free water in the tissues is the cause of fluctuation of the drip.

SUMMARY

A new method for determining drip of frozen products was developed using petroleum ether or Skelly Solve B previously saturated with water as the thawing medium.

The physical and chemical properties of the drip obtained by the new method are stable, and reproducible values are obtained on duplicate samples. Such values can, therefore, be used for the quality control of various frozen foods.

Rapid freezing decreases the quantity of drip and gives a better frozen pack with many products. For products, however, with high bound-water content rapid freezing is not as beneficial as with products of low bound-water content. A definite relationship exists between the drip of various products and their bound-water content.

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SIGNIFICANCE OF BACTERIA IN FROZEN VEGETABLES [1]

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The preservation of food by freezing depends upon retarding the rate of microbial, enzymatic, and chemical changes. It is generally agreed that frozen food should be stored at —17.8°C.(0°F.) or lower to retard such changes. The presence of microorganisms in frozen food may not be important from a health viewpoint, but their presence may be important in determining the storage life of the food and is an indication of the method of handling.

Tressler (1938) considered numbers of bacteria present, ascorbic acid content, and presence of oxidizing enzymes as three important criteria of quality in frozen vegetables. Wide variations in microbial content of vegetables have been reported by Prescott, Bates, and Highlands (1932); Smart (1934, 1937, and 1939); Smart and Brunstetter (1936, 1937); Lochhead and Jones (1936); Diehl, Campbell, and Berry (1936); and Morris and Barker (1935). In correlation studies of a modified plate method and the standard Petri plate method Nickerson (1943) has also shown a wide variation in counts from frozen vegetables.

Although noting a high mortality of bacteria during blanching, Diehl, Campbell, and Berry (1936) and Smart (1939) observed that the blanched vegetable was more subject to rapid growth of bacteria than the raw vegetable. Smart (1939) and Berry (1933) studied types of microorganisms present in the frozen product.

Literature reviews on the microbiology of frozen food have been presented by James (1932), Wallace and Tanner (1933), Berry and Magoon (1934), and Prescott and Tanner (1938).

During the course of several years numerous samples of frozen foods have been examined, from those packed under the most careful conditions to those which were obviously contaminated and in some cases had not been frozen properly. An attempt was usually made to explain excessive counts by a study of contamination of processing equipment or growth of bacteria during handling of the food.

All bacteriological counts were made upon representative samples of raw or frozen vegetables by the usual dilution and plating technic, using a tryptone-yeast extract-glucose agar. Plates were incubated at either 25°C.(77°F.) for three days, 30 to 32°C.(86 to 89.6°F.) for two days for normal counts, or at 45 and 55°C.(113 and 131°F.) for thermophilic counts.

RESULTS AND OBSERVATIONS

Typical samples of good-quality frozen vegetables (Tables 1 and 2) show plate counts as low as 1,200 per gram, and with the exception of corn

TABLE 1

Plate Counts on Quick-Frozen Vegetables

Vegetable	Held at −21.7 to −22.8°C (−7 to −9°F.)		Held at −24.4 to −27.8°C. (−12 to −18°F.)		Held at −40°C (−40°F.)
Broccoli	33[1]	28[1]	....	....	....
Broccoli	43	95	33	36	....
Broccoli	28	15	....	....	....
Broccoli	....	....	19	38	....
Broccoli	17	....	43	....	....
Broccoli	....	....	93	....	322
Broccoli	17	10	73	....	....
Peas	63	31	26	106	112
Lima beans	28	37	64	....	....
Lima beans	....	....	45	86	....
Lima beans	30	36	88	....	....
String beans	1	....	5	3	4
Spinach	145	43	37	....	18
Spinach	14	144	48	....	....
Corn, cut	178	196	196	330	....
Corn, cut	212	136	207	....	....
Spinach	4	13	....	....	....

[1] Plate counts given in thousands per gram.

TABLE 2

Bacterial Plate Counts From Two Series of Good- and Poor-Quality Frozen Peas

Sample	No.	Plate count (thousands per gram)
Good-quality	1	150
	2	162
	3	206
	4	152
	5	190
	6	230
	7	182
	8	92
Poor-quality	9	6
	10	7
	11	11
	12	8
	13	10
	14	7
	15	8
	16	7
Good-quality	1A	420
	2A	297
	3A	116
	4A	286
Poor quality	5A	3
	6A	25
	7A	9
	8A	4

and peas few were above 100,000 per gram. Counts on four lots of peas, two of good quality and two of poor quality, were rather surprising in view of the fact that the series of superior peas yielded higher counts than the inferior peas, averaging 190,000 and 8,700 per gram, respectively. This is understandable when it is realized that the superior peas were tender and thus more subject to bruising and exuding of juice on the belt lines, causing greater subsequent bacterial growth as well as contamination of the pack. On the other hand, these inferior peas were overmature and actually should not have been used for freezing.

Vegetables, as harvested, are highly contaminated with microorganisms. They are usually blanched before packaging and freezing, and it is generally known that a high percentage of the microorganisms are killed during the blanching process. However, the number that survive and the rate at which they multiply after heating and before and during freezing, are important factors in determining the number of bacteria which may ultimately be present.

The two lots of peas studied were divided into several aliquots after washing and sorting. Some of the peas from each aliquot were quick-frozen immediately, and the rest were stored at 1.1 and 21.1°C. (34 and 70°F.). From these, more peas were packaged and frozen at 5, 10, and 20 hours. Similar lots of peas were blanched for five minutes at 82.2°C. (180°F.), 15 seconds at 100°C. (212°F.), 30 seconds at 212°F., and one minute at 212°F. These were cooled immediately and some from each lot packaged and quick-frozen immediately, while some were stored and later packaged and frozen as above. Platings were made for bacterial counts immediately before freezing and after 38, 140, and 288 days.

The results (Table 3) seem somewhat confusing in certain respects. Unblanched peas naturally yielded a higher count than blanched peas; however, the relative increase in count from unblanched peas during holding prior to freezing was not as great as from blanched peas. These increases in count were greater in samples stored at room temperature than those held at 34°F. and, of course, the longer the peas were held the higher the count. However, some samples showed a very marked increase between the first plating and subsequent platings. For example, Sample A2, 0 time, increased from 8,000 in the first plating to 264,000 after 38 days and 400,000 after 140 days. Other samples showed similar increases and still others decreased; B5, 20 minutes, decreased from 408,000 in the first plating to 10,000 in the second. These variations are greater than might be expected from sampling and counting errors. A certain decrease is due to death during freezing storage, particularly among the higher count samples held for five hours before freezing, and those samples held at 34°F. for 10 and even 20 hours. The relative increase in numbers of bacteria during holding is just as great as, if not greater than, occurs in unblanched peas.

The series was repeated, omitting the last blanching but plating samples immediately before and after freezing. Again, very low counts were obtained from blanched samples (Table 4), and the count did not rise greatly in peas held at 34°F. for five hours. Markedly higher counts were obtained from all samples held at 70°F. before freezing. As in the first

series, some of the counts, particularly the higher ones held 21 hours before freezing, increased markedly after freezing. Microscopic examination of some of these indicated that this was due to breaking up of bacterial

TABLE 3

A Study of Bacterial Counts in Blanched Peas

Sample No.	Blanch	Peas held prior to freezing at	Time of plating	Number of hours peas were held before quick-freezing			
				0	5	10	20
		°F.	days				
A2	None	34	Before freezing	8[1]	112	500	296
			38	264	144	350	552
			140	400	112	448	144
			288	142	26	45	47
A3	None	70	Before freezing	....	112	208,000	16,000
			38	....	1600	20,000	....
			140	....	3000	26,400	....
			288	....	100	....	....
B5	5 min., 180°F.	34	Before freezing	0.024	0.24	1.34	408
			38	1.42	3.24	3.20	10
			140	0.40	1.68	2.40	5
			288	0.80	1.24	1.80	6
B6	5 min.	70	Before freezing	....	1.5	58	6,400
			38	....	9.0	63	40,000
			140	....	7.4	72	40,000
			288	....	2.0	15	160,000
C8	15 sec., 212°F.	34	Before freezing	0.016	0.22	2.0	240
			38	10.0	0.60	6.0	....
			140	....	3.00	3.6	24
			288	12.0	....	5.2	4
C9	15 sec.,	70	Before freezing	....	0.96	108	3,040
			38	....	11.20	80	40,000
			140	....	3.60	10	30,000
			288	....	1.68	6	44,000
D11	30 sec., 212°F.	34	Before freezing	0.06	0.23	0.2	....
			38	5.00	0.66	35.2	....
			140		4.66	16.8	....
			288	....	....	57.6	....
D12	30 sec., 212°F.	70	Before freezing	....	0.16	25.2	2,480
			38	....	2.24	17.6	12,800
			140	....	4.40	19.2	51,200
			288	....	2.44	16.0	14,000
E14	1 min., 212°F.	34	Before freezing	0.016	0.184	0.278	164
			38	2.700	0.760	26.0	....
			140	1.840	....	23.0	20
E15	1 min., 212°F.	70	Before freezing	....	0.50	7.6	1,308
			38	....	2.66	11.0	11,200
			140	....	2.40	8.8	8,000

[1] Plate counts given in thousands per gram of peas.

clumps during freezing. Clumping or massing of bacteria was particularly noticeable in Sample B6, held 21 hours before freezing, but it could not be observed in the sample after freezing. This factor, as well as variations in sampling, may account for many of the apparent differences in counts.

These results show that blanched and frozen peas, if not further contaminated, may have a low count but that the number of microorganisms may rise rapidly if the peas are held at room temperature before freezing. This factor is extremely important in commercial processing. The colder the peas, the slower this increase will be.

TABLE 4

A Study of Bacterial Counts in Blanched Peas

Sample No.	Blanch	Storage temperature	Time of plating	Number of hours peas were held before quick-freezing			
				0	5	10	20
		°F.	*days*				
A2	None	34	Before freezing	367¹	288	624	324
			2	296	164	21,200	292
			35	288	108	720	440
			75	88	84	120	84
A3	None	70	Before freezing	....	3,520	240,000	....
			2	....	3,080	2,920,000	....
			35	....	560	2,300,000	....
			75	....	420	2,160,000	....
B5	5 min., 82°C.	34	Before freezing	3.2	60	336	58
			2	7.2	13	10	100
			35	20	4	25	12
			75	24	5	10	11
B6	5 min., 82°C.	70	Before freezing	....	60	400,000	....
			2	....	62	6,000,000	....
			35	....	28	3,040,000	....
			75	....	16	6,400,000	....
D11	30 sec., 100°C.	34	Before freezing	6.8	....	27	26
			2	1.7	43	22	160
			35	1.0	44	36	13
			75	3.2	6	20	4
D12	30 sec., 100°C.	70	Before freezing	....	6	320,000	....
			2	....	5	850,000	....
			35	....	4	730,000	....
			75	....	1.4	400,000	....

¹ Plate counts given in thousands per gram of peas.

Contamination in Processing and Growth During Holding and Freezing: In the commercial processing of vegetables prior to freezing it is impossible to avoid some contamination and some time will elapse between the time of blanching and freezing. Growth of microorganisms will occur and it is therefore essential to reduce the time of processing and degree of contamination. A study of processing lines shows that these factors cannot be avoided but can be minimized.

A study of the processing equipment for corn showed several places where bacteria might multiply and contaminate the pack. Plate counts of corn (Table 5) showed that blanching killed at least 97 per cent of the microorganisms, in this instance leaving only 700 per gram. However, counts obtained in the cooling vat, 10,000 to 18,000, and a very high count, 400,000, obtained from corn around the cutter, indicated contamination and growth. The latter does not represent corn moving along but rather

residue around the cutter which may serve as a source of contamination. After blanching, cooling, cutting, and screening, corn showed an increased count, 17,000 and 31,000 per gram. At the end of the sorting and packing belt, counts of 240,000 and 260,000 per gram were obtained, and counts in this range were obtained from several packages. This indicates a high degree of contamination, possibly from the sorting belts. Plate counts made from scrapings from one of these belts were beyond the dilutions used, possibly in the hundred millions per gram.

TABLE 5

Bacterial Plate Counts on Samples of Corn From Processing Equipment

Source of sample	Approximate count per gram
From husker before washing	72,000
From husker after washing	22,000
After steam-blanching 6½ min., cooled	700
Water from cooling vats—head end	18,000
Water from cooling vats—foot end	10,000
Corn around cutter	400,000
1. Cut corn after freezing	200,000
1a. Cut corn after freezing	77,000
2. Cut corn—early run	350,000
2a. Cut corn—early run	480,000
3. Cut corn—late run	360,000
3a. Cut corn—late run	160,000
4. Cut corn from reels	17,000
4a. Cut corn from reels	31,000
5. Cut corn—end of packing belt	240,000
5a. Cut corn—end of packing belt	260,000
1. Cob corn after packing	33,000
2. Cob corn in 3°F. room, 1 hour	83,000
3. Cob corn in 33°F. room, 3 hours	220,000
4. Cob corn in 33°F. room, 5½ hours	283,000
5. Cob corn in 70°F. room, 1 hour	140,000
6. Cob corn in 70°F. room, 3 hours	2,000,000
7. Cob corn in 70°F. room, 5½ hours	14,000,000

Corn on the cob presents a problem in that it is difficult to cool the cobs after blanching, and thus freezing may be relatively slow even in a quick-freezer. Typical counts obtained by weighing out five grams of kernels of corn, taken at random after freezing, show counts well up in the thousands per gram (Table 5). Some increase is observed in packages held at 0.6°C.(33°F.), and a considerable increase in packages held at 70°F.

A string bean processing line, in which the beans were cut while warm, showed a high degree of thermophilic contamination around the knives, rubber roller, and elevator (Table 6). Counts in the millions were obtained from scrapings and residue around these places, even after washing.

A study of a pea processing line has shown similar results, that is, a high degree of contamination from the sorting belt. It was difficult to collect samples from this line, but effects of this contamination were readily shown in plate counts on the frozen peas.

Peas obtained from another processing line showed the same contamination but to a lesser degree. By the time the blanched peas reached the sorting table the bacterial plate counts were 7,000, 8,000, and 8,600 per gram. After sorting the peas, counts of 24,800 and 26,800 were obtained. The packaged peas varied from 21,000 to 92,000 per gram. There is little doubt that the contaminating organisms are in a growing state and therefore will continue to grow in the peas unless cooled immediately. This rate of growth was demonstrated by holding the peas at 70°F. and plating three of these samples at about two-hour intervals. The plate counts showed that the bacteria practically doubled every hour (Table 7). In other words, the increase began immediately without going through the typical lag phase.

TABLE 6

Thermophile Count From Scrapings From Bean Line
After Blanching

Source of sample	No.	45°C.(113°F.) count	55°C (131°F.) count
Elevator to hopper	1	3,000,000	6,000
	2	2,400,00	....
	3	10,000,000	600,000
	4	20,000,000	1,000,000
	5	21,000,000	800,000
	6	18,000,000	1,100,000
Rubber roller	1	1,000,000	3,200
	2	1,100,000	3,000
	3	120,000	400
	4	100,000	400
	5	6,000	600
	6	8,000	400
	7	1,200,000	1,800
	8	1,200,000	2,800
Knives	1	32,000	8,000
	2	56,000	6,600
	3	20,000	12,000
	4	28,000	7,000
	5	320,000	24,000
	6	520,000	10,000

TABLE 7

Bacterial Plate Counts From Three Packages of Peas Held at
21.1°C.(70°F.) Prior to Freezing

Time		Plate count per gram		
hr.	min.			
....	40	21,000	34,000	8,000
2	40	72,000	49,000	21,000
4	25	297,000	158,000	36,500
6	0	585,000	465,000	90,000
8	0	3,100,000	2,150,000	1,070,000
11	25	21,000,000	7,640,000	26,000,000

It has been shown that bacteria will develop upon processing equipment during operation and that they will contaminate the pack to a limited extent. If one studies such equipment, it becomes obvious that such contamination cannot be entirely avoided. In itself, the contamination that has been observed may increase the bacterial count but it does not necessarily produce a noticeable effect upon quality of the food. However, the higher the count, the closer one approaches the point where bacteria may affect the product. If the food is not frozen properly, bacteria may multiply excessively and cause a noticeable effect.

TABLE 8

Summary of Bacterial Plate Counts [1] on Frozen Peas

Laboratory samples; washed and packaged immediately	Washed and packaged; peas dry	Little contamination from equipment		Contaminated from equipment		Contaminated from equipment; possibly some growth before freezing		Contaminated and frozen improperly; definite indication of growth in package		Frozen improperly; peas show gross spoilage	
0.8	6.0	81	22	190	400	660	910	1300	2500	45,000	22,000
1.8	7.0	28	21	213	420	710	840	4000	1600	118,000	26,000
0.5	11.0	79	43	240	150	760	770	2400	1000	95,000	32,000
1.7	8.0	78	50	400	136	790	840	1600	2400	35,000	13,000
0.4	10.4	80	58	140	320	510	720	2200	3200	13,000	340,000
0.3	7.4	73	48	200	460	850	900	2600	3400	25,000	1,000,000
0.8	8.0	15	86	163	330	920	......	6600	6880	45,000	1,000,000
0.3	7.6	18	30	121	110	800	......	1090	2570	14,000	4,000,000
0.1	3.0	14	30	100	136	870	......	1260	1440	14,000	....
0.2	25.0	45	60	116	180	910	......	1450	2900	12,000	....
2.5	9.0	46	51	105	240	820	......	3100	1030	13,000	... .
11.0	4.0	19	24	100	430	860	......	2700	8100	17,000	....
3.1	......	9	65	142	......	860	......	1100	4000	20,000	....
1.3	......	20	63	100	......	680	......	1370	5400	92,000	....
0.7	......	19	31	101	......	560	......	1260	3600	78,000	... .
0.5	......	20	26	270	......	630	......	1350	5400	57,000	....

[1] Estimated counts given in thousands per gram.

In commercial freezing of vegetables after the package is filled, it may be an hour or more before it can be frozen. It has been pointed out that the bacteria will then multiply quite rapidly. A number of packages of peas which had been packed in cartons and placed in the freezer room, but owing to poor air circulation did not freeze uniformly, were made available for study. There is little doubt that growth had occurred after packaging. The counts on these, as well as counts on properly frozen peas, have been compared (Table 8) and grouped. The first group were laboratory samples, that is, they were blanched, cooled, and hand-packed. Counts were naturally low. The second group of 12 represents counts from overmature frozen peas upon which bacteria would not grow rapidly.

There seems to be little doubt that those 32 commercial samples in the next group with counts below 100,000 (Table 8), the majority of which are below 30,000, represent a condition in which a minimum contamination and growth had occurred. These might be considered as average counts obtained under good commercial conditions. Another group of 29 samples

had plate counts between 100,000 and 500,000, the majority in the lower range. This seems to be associated with a gross equipment contamination, or some growth in the package prior to freezing. Another group of 22 samples yielded counts from 500,000 to 1,000,000. These, and those samples in the next two groups of 56 samples with counts of over a million, seem to represent that condition in which gross contamination was followed by growth in the package. The majority of these samples were definitely inferior and showed the detrimental effects of bacterial growth. Some of these had actually become brown in color and putrid in odor, and it is doubtful if they were frozen for at least a day after packaging.

DISCUSSION AND SUMMARY

In the processing of vegetables for freezing, a large proportion of the bacteria present on the vegetables is killed in the blanching process. However, bacteria may develop during the cooling, handling, and prefreezing period.

Under commercial conditions some contamination of the food by bacteria occurs, particularly from places on which juice of the vegetable is exuded and serves as a bacterial growth medium.

An important fact which is usually overlooked is that these contaminating microorganisms are in a rapid growth phase rather than a lag phase. Chesney (1916) pointed out that organisms will continue growth at the same rate when transferred to a new medium. This being the case, growth of these contaminating organisms will continue at a rapid rate unless it is stopped quickly by subjecting the food to low temperatures. With a low degree of contamination, for example, 10,000 per gram, a count of a million or more may be attained within a few hours. If these organisms were in a lag phase or had been recently subjected to a heat shock, however, more than that period of time would be required for growth actually to get under way. Therefore, we must consider that the organisms introduced by growth in exudates on equipment are far more subject to yielding high counts in foods than are those present on the vegetables.

Bacterial counts should not be thought of as an indication of the quality of the food but rather as an indication of handling of the food between blanching and final freezing. Very low counts are an indication of properly blanched food and extreme cleanliness of equipment. Contamination from equipment will cause somewhat higher counts but the very high counts, that is, a million or more per gram, undoubtedly are the result of equipment contamination followed by too-long holding before freezing.

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EFFECT OF CERTAIN HOME PRACTICES ON REDUCED ASCORBIC ACID CONTENT OF PEAS, RHUBARB, SNAP BEANS, SOYBEANS, AND SPINACH [1]

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These experiments were undertaken in order to study the extent to which the reduced ascorbic acid content of five vegetables was affected by several common procedures. The home practices investigated were holding freshly harvested snap beans at room temperature and in the refrigerator; boiling fresh peas, snap beans, soybeans, and spinach in different amounts of water for various lengths of time; cooking and holding rhubarb; and cooking, holding, and reheating spinach.

There are numerous reports in the literature dealing with the ascorbic acid content of raw peas, rhubarb, snap beans, and spinach and the effects of storage, cooking, and holding and reheating on the amounts present. No attempt has been made here to review all the articles that have been published on these subjects; a compilation of published data on the amounts of ascorbic acid retained when raw peas, rhubarb, snap beans, soybeans, and spinach were cooked is presented (Table 1). Some of the tabulated results and some from other investigations will be considered later, in detail, in relation to those obtained in the present study.

EXPERIMENTAL PROCEDURE

The Dark Podded Thomas Laxton peas, Victoria rhubarb, Bountiful and Stringless Black Valentine snap beans, Iligan soybeans, and Blight Resistant Savoy spinach used in this study were grown under the supervision of the Department of Horticulture, University of Illinois. The vegetables were harvested at suitable stages of maturity for eating and were brought to the food research laboratory on the day they were picked. Preparation of the vegetables was started immediately. Snap beans were obtained from different plantings in 1943—Bountiful snap beans, from June 30 to July 7, and Stringless Black Valentine snap beans, from August 5, to September 9. In the same year, work was done on Iligan soybeans from September 20 to 24 and on Blight Resistant Savoy spinach from October 19 to 29. Dark Podded Thomas Laxton peas were obtained from June 12 to 15 in 1944, and Victoria rhubarb from May 14 to 22 in 1945. The effect of the following procedures was investigated.

Holding at Different Temperatures: One picking of Bountiful snap beans was subdivided into three lots and each of the three pickings of

TABLE 1

*Retention of Ascorbic Acid When Raw Peas, Rhubarb, Snap Beans,
Soybeans, and Spinach Were Cooked*

Vegetable, variety (if known), and cooking procedure	Ascorbic acid retained in cooked vegetable	Reference
	pct.	
Peas:		
350 gm., 3 cups water, 20 to 30 min., open kettle	46	Brinkman *et al.* (1942)
350 gm., waterless cooker	48	Brinkman *et al.* (1942)
350 gm., pressure saucepan at 15-lb. pressure	58	Brinkman *et al.* (1942)
Cooked to give a palatable product	57	Burrell and Ebright (1940)
Thomas Laxton, 525 gm., 700 ml. water, approximately 2 min. back to boiling, boiled 14 min	42	Fenton *et al.* (1936)
Alderman, 525 gm., 700 ml. water, approximately 2 min. back to boiling, boiled 13 min	53	Fenton *et al.* (1936)
Cooked - no details given	39	Harris and Olliver (1942)
85 gm., 180 ml. water, 17 min	45-60	Johnston *et al.* (1943)
Boiling (14 samples, 3 experiments)	62	Lampitt *et al.* (1943)
Boiling	38	Olliver (1940)
Boiling, 1 part vegetable to 3 parts water, 25 min	37-42	Olliver (1941)
454 gm., 15 ml. water, 5 min. to boiling, 15 min. simmer	86	Oser *et al.* (1943)
454 gm., 360 ml. water, 4 min. to boiling, 20 min. simmer	68	Oser *et al.* (1943)
Laxtonian, boiled 30 min., open kettle	67	Richardson and Mayfield (1940)
Thomas Laxton, 350 gm., 120 ml. water, 4 to 5 min. to 99°F., cooked 8 min	71	Todhunter and Robbins (1941)
Alderman prepared as above	76	Todhunter and Robbins (1941)
Thomas Laxton, 350 gm., 350 ml. water, 5 to 5.5 min. to 99°F., cooked 8 min	54	Todhunter and Robbins (1941)
Alderman prepared as above	57	Todhunter and Robbins (1941)
Thomas Laxton, 350 gm., steamed 6.5 min. to 99°F., cooked 8 min	80	Todhunter and Robbins (1941)
Alderman prepared as above	86	Todhunter and Robbins (1941)
Rhubarb:		
Victoria, peeled, cut in 0.5 inch pieces, 400 gm., 30 ml. water, cooked 10 min; first 2 min. in high, Pyrex pan	86	Brown *et al.* (1941)
As above in covered Pyrex pan	92	Brown *et al.* (1941)
4 parts sliced rhubarb, 1 part water, 2 parts sugar, boiled for 5 min	60-70	Clague *et al.* (1935)
Snap beans:		
255 gm., beans cut in 2 or 3 pieces, 1,020 ml. water, 30 to 35 min., open kettle	51	Brinkman *et al.* (1942)
255 gm., waterless cooker	49	Brinkman *et al.* (1942)
255 gm., pressure saucepan	40	Brinkman *et al.* (1942)
Cooked to give a palatable product	73	Burrell and Ebright (1940)
Bountiful, 4 parts beans to 1 part water, 30 min	76	Farrell and Fellers (1942)
255 gm., 1,020 ml. water, 30 min	42	Halliday and Noble (1936)
Cooked—no details given	42	Harris and Olliver (1942)
255 gm. whole, 1,020 ml. water, 30 min., open kettle	58	Ireson and Eheart (1944)
255 gm. whole, 40 ml. water, 30 min., covered	59	Ireson and Eheart (1944)
Refugee, 255 gm., 1,020 ml. water, 2 min. to boiling, boiled 15 min	62	Mack *et al.* (1939)
Kentucky Wonder, 255 gm., 1,020 ml. water, 2 min. to boiling, boiled 17 min	66	Mack *et al.* (1939)
Soybeans:		
Jogun, cooked 25 min	100	Reid (1943)
Spinach:		
Steam cooked, 1 lb., using 100 ml. water	68	Dunker and Fellers (1938)
Water cooked, 1 lb. to 900 ml. water	33	Dunker and Fellers (1938)
Heavy pack, garden fresh, 300 gm., 600 ml. water, 2 min. to boiling, boiled 6 min	24	Gleim *et al.* (1944)
Heavy pack, garden fresh, 300 gm., 60 ml. water, 2 min. to boiling, boiled 6 min	62	Gleim *et al.* (1944)
570 gm. wet spinach in 2.5 cups water, 8 min	24	Halliday and Noble (1936)
Cooked—no details given	41	Harris and Olliver (1942)
1 part spinach, 1 part water, 7 min., open kettle	39	Hollyman *et al.* (1944)
In water clinging to leaves, 8 min., open kettle	57	Hollyman *et al.* (1944)
60 ml. water, pressure saucepan, 0.5 min. at pressure	48	Hollyman *et al.* (1944)
In water clinging to leaves, pressure saucepan, 0.5 min. at pressure	51	Hollyman *et al.* (1944)
Boiling (9 samples, 9 experiments)	37	Lampitt *et al.* (1943)

Stringless Black Valentine snap beans into four lots. Moisture and ascorbic acid determinations were made within approximately two hours after harvesting, after holding for different periods at room temperature [25 to 28°C.(77 to 82.4°F.)], and in the refrigerator [2 to 9°C.(35.6 to 48.2°F.)]. The lots of beans which were held were packaged in one thickness of wrapping paper.

Variations in Amounts of Water Used and Time of Boiling: Three hundred-gram portions of Dark Podded Thomas Laxton peas, Bountiful snap beans, and Iligan soybeans were boiled in half and twice their weight of water for the shortest periods of time required to give tender "done" products. The vegetables were also boiled in twice their weight of water for twice the minimum boiling times; lengths of the boiling periods are given (Table 3). Two-quart aluminum pans with fitted covers were used. Preparatory to cooking, snap beans were cut in one and one-half inch pieces, and 1,800 grams of soybeans were blanched in six quarts of boiling water for five minutes, cooled, and hulled. Details of the cooking procedure—regulation of gas burners, control of amounts of water, amounts of salt used, and draining and weighing of the cooked products—were carried out according to the mimeographed outline of Recommended Laboratory Procedures for Food Preparation and Food Preservation for the National Cooperative Experiment Station Project on "Conservation of Nutritive Value of Foods." Four hundred-gram portions of Blight Resistant Savoy spinach were cooked in three-quart aluminum pans with fitted covers in the water clinging to the leaves after washing, in half their weight of water, and in twice their weight of water for seven minutes. In the case of spinach this length of time is the total cooking time including that required for boiling or steaming to be resumed after the vegetable was added. The Recommended Laboratory Procedures were modified in that one and one-half grams of salt were used when spinach was cooked in the water clinging to the leaves and that all cooked samples were drained for three minutes and turned with a fork during the process. Four extractions and subsequent determinations of ascorbic acid were made on all raw and cooked portions of these vegetables and each experiment was repeated four times. Two aliquots of each cooking water were used for ascorbic acid determinations.

Cooking, Holding, and Reheating: Three lots of Blight Resistant Savoy spinach were cooked as before in the water clinging to the leaves. One was sampled immediately after draining, while the others were allowed to cool for one hour and held in covered containers in the refrigerator for 23 hours. After holding, another lot was sampled for ascorbic acid determinations and the third lot was boiled in 100 ml. of water for one minute, drained, and sampled. The experiment was repeated four times.

Approximately 2,000 grams of Victoria rhubarb were peeled, cut in one-inch pieces, thoroughly mixed, and four 400-gram lots were weighed out. One was sampled immediately, and the others were held in the refrigerator in covered containers for short periods of time until they were cooked. Four hundred grams of rhubarb were cooked in 30 ml. of water in a two-quart, covered Pyrex saucepan over a moderately low flame for 12 minutes. The rhubarb was stirred occasionally with a glass stirring

rod which was placed through the pouring spout. At the end of the cooking period the sauce was poured over 150 grams of sugar in an enamel bowl and the mixture was stirred three minutes to dissolve the sugar. The lots of cooked rhubarb were sampled at once after being allowed to cool for one hour and after holding in the refrigerator for 23 and 71 hours. This experiment was repeated four times. Preliminary to this experiment peeled, sliced rhubarb was held in covered containers in the refrigerator and sampled for ascorbic acid determinations before holding and after one, two, and four hours.

Reduced ascorbic acid was determined by the titration method of Tillmans, Hirsch, and Hirsch (1932) as modified by Bessey and King (1933) and Bessey (1938). The detailed procedures were similar to those reported by Van Duyne, Chase, Retzer, and Simpson (1945) for all vegetables except rhubarb. Samples of rhubarb weighing 150 grams or of sauce weighing 200 grams were ground with three per cent metaphosphoric acid in the Waring blendor; the mixture was transferred to a volumetric flask and made up to volume. Ascorbic acid was extracted as before from aliquots of this volume.

TABLE 2

Ascorbic Acid Content of Snap Beans Within Two Hours After Harvesting and After Holding in Refrigerator and at Room Temperature

Variety of snap beans	Treatment	Number of determinations	Ascorbic acid mean and standard deviation of mean fresh weight	Ascorbic acid mean and standard deviation of mean dry weight
			mg./gm	mg./gm.
Bountiful	Within 2 hours after harvesting	4	.22±.013	2.6±.13
	Held 25 hours at room temperature	6	.16±.005	1.8±.06
	Held 2 hours at room temperature and 23 hours in refrigerator	6	.20±.005	2.5±.07
Stringless	Within 2 hours after harvesting	16	.20±.012	2.3±.11
Black	Held 24 hours at room temperature	16	.14±.008	1.6±.09
Valentine	Held 24 hours in refrigerator	16	.20±.014	2.5±.17
	Held 48 hours in refrigerator	16	.18±.012	2.2±.12

RESULTS AND DISCUSSION

Effect of Holding at Different Temperatures: Values for the ascorbic acid content of freshly harvested and stored Bountiful and Stringless Black Valentine snap beans are given (Table 2). No significant differences [3] were found between the amounts of ascorbic acid present within approximately two hours after picking and after holding the beans for approximately one or two days in the refrigerator. Holding the beans at room temperature, however, resulted in significant decreases in the ascorbic acid content. Compared on the dry-weight basis Bountiful snap beans lost 31 per cent of their ascorbic acid content and Stringless Black Valentine snap beans lost 30 per cent in 25 and 24 hours, respectively. After four days of

[3] A difference is considered significant if it is three or more times the standard error of that difference.

storage at room temperature, Peterson (1946) found that the amount of ascorbic acid present in Tendergreen snap beans had decreased only 20 per cent. Zepplin and Elvehjem (1944) reported that green beans lost 20 per cent of their ascorbic acid when held for 24 hours at room temperature and 10 per cent if kept on the shelf in a mechanical refrigerator for 24 or 48 hours. Percentage losses in ascorbic acid content ranging from 24.4 to 30.8 were found by Harris, Wissmann, and Greenlie (1940) to result from three days of storage in different refrigerators. The more extensive work of Mack, Tapley, and King (1939) on the effect of storage on the ascorbic acid content of four varieties of snap beans held at three temperatures showed that in some instances variety might be a more important factor than temperature in determining the amounts retained. Hence, the variations in the percentage retentions reported by different workers are to be expected.

Effect of Variations in Amounts of Water and Time of Boiling: Mean figures for the ascorbic acid content of freshly harvested peas, snap beans, soybeans, and spinach; for the ascorbic acid content of these vegetables after cooking in different ways; and for the percentage retentions of ascorbic acid in the cooked vegetables and in the cooking waters are presented (Table 3). When 300 grams of Dark Podded Thomas Laxton peas were boiled in 150 ml. of water for 15 minutes, 74 per cent of the ascorbic acid content was retained. This percentage retention is higher than the majority of figures reported in the literature (Table 1). However, it is comparable to the values obtained by Todhunter and Robbins (1941), when peas were cooked in small amounts of water, and lower than the 86 per cent retention reported by Oser, Melnick, and Oser (1943) for peas cooked in a minimum amount of water. In the present study the ascorbic acid content of peas cooked in twice their weight of water was significantly lower than of those boiled in the smaller amount. A further small but significant decrease in the amount of ascorbic acid present occurred when the cooking time was doubled.

Bountiful snap beans retained 78, 66, and 56 per cent of the original ascorbic acid content when this vegetable was boiled in half its weight of water for 15 minutes, in twice its weight of water for 15 minutes, and in twice its weight of water for 30 minutes, respectively. In general, these results are similar to those obtained by other workers when comparable cooking practices were used. Ireson and Eheart (1944) found no significant difference between the ascorbic acid content of whole snap beans cooked for 30 minutes in 1,020 ml. of water in an open kettle and of those cooked for the same length of time in 40 ml. of water in a covered kettle. From the results obtained in the present study a greater loss in ascorbic acid content would be expected when the volume of the cooking water was increased. However, details of the cooking procedure differed in the two series of experiments.

Depending upon the amounts of water and the lengths of the boiling periods used, cooked Higan soybeans contained 0.23 ± 0.004, 0.16 ± 0.002, and 0.14 ± 0.004 milligram of ascorbic acid per gram. The largest per cent retention obtained, 71 per cent when soybeans were boiled in half their

TABLE 3

Effect of Boiling in Different Amounts of Water for Various Lengths of Time on Ascorbic Acid Content of Vegetables

Vegetable	Raw		Details of cooking procedures			Ascorbic acid			
	Moisture mean and standard deviation of mean	Ascorbic acid mean and standard deviation of mean	Weight of vegetables taken for cooking	Amount of boiling water used	Length of boiling period	In cooked vegetable as determined mean and standard deviation of mean	Calculated on raw-weight basis [1]		
							Retained in cooked vegetable	In cooking water	Lost
	pct.	*mg./gm.*	*gm.*	*gm.*	*min.*	*mg./gm.*	*pct.*	*pct.*	*pct.*
Peas	79.1±.54	.24±.007	300	150	15	.19±.004	74	19	7
			300	600	15	.13±.000	50	44	6
			300	600	30	.12±.002	48	52	0
Snap beans	91.3±.28	.24±.008	300	150	15	.20±.004	78	9	13
			300	600	15	.16±.005	66	12	22
			300	600	30	.13±.005	56	18	26
Soybeans	66.3±.24	.31±.006	300[2]	150	12	.23±.004	71	12	17
			300[2]	600	12	.16±.002	52	26	22
			300[2]	600	24	.14±.004	45	36	19
Spinach	91.9±.17	.65±.012	400	.....[3]	7[4]	.59±.020	58	15	27
			400	200	7[4]	.47±.011	47	24	29
			400	800	7[4]	.36±.015	36	28	36

[1] In the case of soybeans the assumption was made that there is no difference between the weights of raw soybeans and those which have been blanched and cooled in the pods. [2] Weight of hulled soybeans used (soybeans were blanched 5 min., cooled, dried, and hulled). [3] Water clinging to leaves after washing. [4] Total cooking time.

weight of water for 12 minutes, is lower than the 100 per cent reported by Reid (1943) for Jogun soybeans cooked for 25 minutes.

Blight Resistant Savoy spinach cooked for seven minutes in the water clinging to its leaves after washing, in half its weight of water, and in twice its weight of water retained 58, 47, and 36 per cent of ascorbic acid, respectively. These results are similar to others reported in the literature. Although spinach loses a higher percentage of its ascorbic acid content than do peas, snap beans, and soybeans when cooked in comparable ways, the amounts of ascorbic acid present in all samples of cooked spinach are greater than those in the other cooked vegetables.

TABLE 4

Effect of Cooking, Holding, and Reheating on Ascorbic Acid Content of Spinach and Rhubarb

Vegetable	Treatment	Moisture mean and standard deviation of mean	Ascorbic acid content mean and standard deviation of mean as determined	Retention of ascorbic acid in vegetable calculated on raw-weight basis
		pct.	*mg./gm.*	*pct.*
Blight Resistant Savoy spinach	Within 2 hours after harvesting............	91.4±.24	.69±.017	....
	Cooked (400 gm. in water clinging to leaves for 7 min.).............................	....	.65±.012	58
	Cooked, cooled 1 hr., 23 hr. in refrigerator.................................	....	.59±.013	52
	Cooked, cooled 1 hr., 23 hr. in refrigerator and reheated............................	....	.41±.008 [1]	33 [1]
Victoria rhubarb	Within 2 hours after harvesting............	94.5±.09	.094±.0049	....
	Cooked (400 gm. in 30 ml. water for 12 min., 150 gm. sugar added).............	....	.059±.0035	87
	Cooked, cooled 1 hr.................................	....	.057±.0028	85
	Cooked, cooled 1 hr., 23 hr. in refrigerator..................................	....	.060±.0027	86
	Cooked, cooled 1 hr., 71 hr. in refrigerator..................................		.058±.0033	83

[1] Mean value obtained from three instead of four experiments.

Effect of Cooking, Holding, and Reheating: The results obtained when Blight Resistant Savoy spinach and Victoria rhubarb were cooked and held and, in the case of spinach, reheated are given (Table 4). The mean value for the percentage retention of ascorbic acid when spinach was cooked for seven minutes in the water clinging to the leaves was 58, the same value that was obtained in the previous series of experiments. There was a slight but significant decrease in the amount of ascorbic acid present when the cooked spinach was cooled for one hour and held for 23 hours in the refrigerator, and only 33 per cent of the original amount was retained after reheating the cooked, held spinach. Hollyman, Brodie, and Willard (1944) reported an average loss of 64 per cent when spinach was reheated for 15 minutes after 24 hours of refrigeration.

In a preliminary experiment rhubarb was peeled, cut into one-inch pieces, and divided into four 400-gram lots. One lot was sampled imme-

diately and the others after holding for one, two, and four hours in the refrigerator. The total ascorbic acid contents of these samples were 29.14, 29.95, 29.07, and 29.95 milligrams, respectively. Since these values are not significantly different, it was decided that holding the peeled, cut rhubarb for short periods before cooking would not influence the final results of the cooking and holding study. Eighty-seven per cent of the original amount of ascorbic acid was retained in the sauce. The amount present in the sauce was not changed significantly by cooling for one hour or by holding for 23 and 71 hours in the refrigerator. The per cent retention on cooking is higher than that of Clague, Fellers, and Stepat (1935) but approximately the same as the retentions determined by Brown, Schuele, and Fenton (1941). It is interesting to note that holding in the refrigerator resulted in no change in the ascorbic acid content of rhubarb sauce and a further loss of six per cent in cooked spinach. The 40-per cent decrease in the ascorbic acid content of potatoes on holding, reported by Van Duyne, Chase, and Simpson (1945), is of much greater magnitude.

SUMMARY

The mean values for the ascorbic acid contents of Bountiful and Stringless Black Valentine snap beans, determined within two hours of harvesting, were 0.22 ± 0.013 and 0.20 ± 0.012 milligram per gram, respectively. The amounts present were not significantly affected by storage for approximately one day in the refrigerator; in the case of Stringless Black Valentine snap beans two days of refrigerator storage were without effect. Calculated on the dry-weight basis Bountiful snap beans, held 25 hours at room temperature, lost 31 per cent of the amount of ascorbic acid originally present and Stringless Black Valentine snap beans, held at room temperature for 24 hours, lost 30 per cent.

The fresh Dark Podded Thomas Laxton peas, Bountiful snap beans, Iligan soybeans, and Blight Resistant Savoy spinach used to study the effect of variations in volume of water and in time of boiling contained 0.24 ± 0.007, 0.24 ± 0.008, 0.31 ± 0.006, and 0.65 ± 0.012 milligram of ascorbic acid per gram, respectively. When peas, snap beans, and soybeans were boiled in twice their weight of water, the amounts of ascorbic acid retained in the cooked vegetables were significantly less than when the vegetables were boiled in half their weight of water. Doubling the cooking times significantly decreased the amounts of ascorbic acid present from the values obtained when the three vegetables were cooked only to "done" stage. Increasing the amounts of water in which spinach was cooked while keeping the cooking time constant, decreased the percentage retentions of ascorbic acid in the cooked vegetable.

The amounts of an average daily allowance of 75 milligrams of ascorbic acid which could be supplied by these cooked vegetables were calculated. One hundred-gram portions of the peas, snap beans, and soybeans which had been boiled in the smaller amounts of water for the shorter lengths of time could supply approximately 25, 27, and 31 per cent, respectively, of such an allowance. Spinach cooked in the water clinging to the leaves for seven minutes would provide approximately 79 per cent in a one hundred-gram portion.

Spinach containing 0.69 ± 0.017 milligram per gram of ascorbic acid retained 58 per cent after being cooked in the water clinging to the leaves for seven minutes; 52 per cent after cooking and holding 23 hours in the refrigerator; and 33 per cent after cooking, holding, and reheating. When rhubarb sauce was made, 13 per cent of the ascorbic acid content was lost. The amounts present in the sauce were not affected by cooling or by holding in the refrigerator for 23 or 71 hours.

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EFFECT OF SOY FLOURS ON RATE OF STALING IN PLAIN CAKE [1]

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It has been suggested by Stellar and Bailey (1938) that soy flour has an ability to retard staling when used in bakery products, but little information is available to indicate the extent to which it is effective for this purpose. An investigation in this connection therefore seemed desirable, and a study was undertaken of the comparative effectiveness of high-fat (20 to 22 per cent fat), low-fat (five to eight per cent fat), and minimum-fat (one per cent fat or less) soy flours in retarding the staling of plain, shortened cake.

REVIEW OF LITERATURE

Numerous studies have been made in an effort to measure the degree of staling in baked products and to study the rate at which the process takes place—Platt (1930), Stellar and Bailey (1938), and Platt and Powers (1940). Several objective methods have been devised for measuring staling in baked products; chief among these are compressibility tests and grain and volume records.

Compressibility Tests: Considerable attention has been given to pieces of apparatus for measuring the compressibility of baked products. Bread and cake, for example, are quite compressible when fresh but are much less so as they become stale.

One of the early devices used for compressibility measurements was that of Katz (1928) in which the descent of a weighted plunger, as it compressed the bread for a period of three minutes, was indicated by means of a pointer. Bailey (1930) also made measurements by allowing a given weight to rest on a prism of bread for a given period of time. About the same time, Platt (1930) made use of this same principle by attaching a plunger to the bottom of one pan of a large analytical balance. The depression of the plunger into a slice of bread was recorded by means of a pointer in front of a millimeter scale.

An apparatus which has been described by King, Morris, and Whiteman (1936) is somewhat different in design but similar in purpose to that used by Platt. In this case, a penetrometer was used for measuring compressibility of the cake. The compressibility was recorded in centimeters from the scale machined on the moveable post which held the disk used to compress the sample.

A Russian worker, Nikolayev (1941), described a somewhat different kind of apparatus used for judging bread "deformation" (compressibility). However, this method also was based on the action of a definite load during a stated period of time. Again, the position of the pointer on the measuring scale was recorded.

Platt and Powers (1940) described still another type of apparatus designed by Baker which permitted the measurement of strain (compression) at a series of stresses.

An interesting departure from the usual methods for measuring the compressibility of bread was devised by Morison and Coriolis (1929), reported by Platt and Powers (1940). In this case, the weight used to compress the bread was many times that reported by any other investigator.. A prism of bread was subjected to a seven-pound weight for 15 seconds. The weight was then removed, the bread was allowed to spring back towards its normal shape for 30 seconds, and the percentage of rebound was calculated.

Permanent Records of Grain and Volume: Symmetry, grain, and volume have been used to denote changes during staling of baked products. It is desirable to have permanent records of these properties in following the staling process. Photography, photographic projections, ink imprints, and preserved bread slices have been used for this purpose.

Matejovsky (1938) has reported making contact prints from thin slices of a baked product directly upon a contrast photographic paper. Cathcart (1938), by passing light through a slice of the baked product, was able to secure the image on photographic projection paper. Cathcart (1939), Heald (1929), Harrel (1930), and Platt and Kratz (1933) have all reported photo-records as a means of obtaining permanent records of baking studies. Lee (reported by Heald in 1929) devised a method of spraying the cut surface of bread with China White before making an exposure. This made the bread appear whiter, thus giving better contrast in the photograph.

In some instances, ink imprints have been used instead of photographs. Mohs (1924) used a paste-like mixture of lamp black and oil to blacken the cut surface of bread. The slice was then pressed exactly like a stamp upon a piece of unsized paper. The resulting impression retained the grain-cell structure in its natural form. Child and Purdy (1926) made prints of cake slices using a mixture of mimeograph ink, glycerine, and water. Grain was easily judged by means of these ink imprints.

Markley (1934), instead of using reproductions for permanent records, described a method whereby the bread samples themselves were preserved as permanent standards.

Subjective Tests: Subjective tests have value in judging food, although they should not be used as the sole criterion of quality, since it is difficult to control adequately all the conditions pertaining to subjective reactions.

King (1937) has drawn attention to the numerous difficulties involved in obtaining a panel of judges. Freeman (1941) brought out the fact that even with a permanent standard it was probable that an experienced judge would not be entirely consistent from time to time. Knowles and

Johnson (1941) have pointed out, also, that since individuals vary widely in their likes and dislikes, as well as in their sensitivity, consistent results are difficult to obtain even under optimum conditions. Thus, it is evident that there is a need for further refinements in technique of performing organoleptic tests in order that the data may be more reproducible.

EXPERIMENTAL PROCEDURE

In this study standard cakes and cakes made with the three types of soy flour were rated by compressibility tests, subjective judging, and ink imprints. The ratings were made on the cakes while fresh and after definite storage periods. Results obtained from the compressibility tests and the subjective judging were statistically evaluated. It was necessary to evaluate the ink imprints subjectively. Following are the details of the procedure which was used.

Preparation of Cakes: The following cake formulas (each formula yielding seven cakes) were used:

	Standard cake	Soy-flour cake*
	gm.	*gm.*
Cake flour	375.00	318.75
Soy flour (high-fat, low-fat, or minimum-fat)		56.25
Sugar	336.00	336.00
Hydrogenated fat	162.00	162.00
Egg	201.00	201.00
Milk	297.00	297.00
Salt	6.00	6.00
Baking powder	15.60	15.60

* The flour used in this formula was 85% cake flour and 15% soy flour, by weight.

The flour, sugar, and salt were sifted. Next, all of the cake ingerdients (except three tablespoons of the combined dry ingredients and the baking powder) were combined in an electric mixer.[3] The reserved dry ingredients were mixed and added during the last few minutes of the mixing period. Aliquots of the batter (186 grams) were weighed into each of seven cake pans (6"x3¼"x2¼") and baked at 176.5° C. (350° F.) for 40 minutes. The cakes were cooled, wrapped well in waxed paper, and placed in 50-pound lard cans with tightly fitting lids for storage until time for the various tests.

Each time that cakes were prepared one formula (yielding seven cakes) was made from each type of soy flour and, in addition, one formula was made from cake flour to serve as the standard. The three soy flours used throughout the study were taken from the same representative lots obtained at the beginning of the study.

Compressibility Test: For use in this laboratory the authors obtained a compressibility apparatus of the balance type of construction similar to the one used by Platt (1930). This apparatus was found rather unsatisfactory for this cake study, since the readings were not in a definite unit of measurement, but instead were indicated as being so many marks to the right or left of the center point on a scale. There was also difficulty

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[3] Hobart Kitchen Aid electric mixer (Model G—three speeds).

in standardizing the apparatus so that the starting position was always the same.

In view of these difficulties a new type of apparatus was designed and constructed for the purpose of measuring the compressibility of the cakes. The chief improvement over some previous pieces of apparatus used for this purpose was that the compressibility readings were obtained in a definite unit of measurement (grams) and that smaller differences in compressibility could be measured. By changing the distance through which a product was compressed, the apparatus was adjustable for use with products having a wide range of compressibility. Tests were made easily and rapidly once the equipment had been adjusted to the particular product being studied.

In principle the method was as follows: a slice of cake of given thickness was compressed through a given distance, and the pressure which had been exerted in the operation was recorded in grams.

The degree of reproducibility of data obtained by use of this machine is indicated by the following figures for compressibility resulting from five standard (cake-flour) cakes three hours after baking:

	Cake 1	Cake 2	Cake 3	Cake 4	Cake 5
	gm.	gm.	gm.	gm.	gm.
Slice 1	70	69	70	68	67
Slice 2	68	68	70	70	68
Slice 3	66	67	68	70	65
Slice 4	68	69	71	70	67
Slice 5	69	71	67	70	71
Average	68.2	68.8	69.2	69.6	67.6

It is interesting to note that the reading for the center slice (Slice 3) was often slightly lower than the readings for the slices nearer the ends of a given cake. This was considered as one indication that reproducible results were being obtained. Evidence that the method of testing was satisfactory was also found in the fact that the figure for the difference between the average readings of any two of the five duplicate cakes ranged from ± 0.6 to ± 2.0 grams. The reproducibility of readings in the above sets of compressibility readings is typical of that obtained throughout the investigation.

Compressibility readings were taken when the cakes were 3, 24, 48, and 96 hours old. For this purpose, cakes were sliced into three-fourths-inch slices by the use of a mitre box. A round metal cutter with a sharp cutting edge was used to cut a piece from each of five center slices used for compressibility readings, and the slices were numbered one to five from one end of the cake to the other. The compressibility reading for any given cake was the average value obtained for the five slices. Readings were made on a total of 16 cakes for each type of flour and at each period of storage. ·

Subjective Judging: The three cakes (from each batch of seven) not used for compressibility determinations were used for subjective judging. The cakes were judged on the day they were baked, also when approxi-

mately 24 and 72 hours old. For this test the cakes were sliced, the slices numbered, and the number of the slice given to any one judge was always the same. There were six judges throughout the experiment with an average number of five for each judging. The judges recorded ratings of each cake on a score card prepared for this particular study. On this score card, texture, moistness, tenderness, grain, color, freshness, and general desirability were rated as very poor, poor, fair, good, or very good. Flavor (extent to which soya flavor was evident) was scored very pronounced, pronounced, definite, scarcely evident, or not evident; degree of acceptability was scored as very objectionable, objectionable, acceptable, pleasing, or very pleasing.

A statistical analysis was made of the ratings of freshness and general desirability to determine which showed significant differences.

Ink Imprints: Five batches of each of the four kinds of cake, baked and handled exactly as in the previous tests, were used for the purpose of making ink imprints. Before making imprints, the cake was frozen for 24 hours in a freezing unit. By recording the imprints immediately after removal from the unit it was possible to obtain a fairly satisfactory picture since there was little loss of crumb from the cut surface of frozen cake. The cake was cut evenly in half, crosswise, by the use of a mitre box. An imprint was made from the cut surface of one half. Imprints were made on cakes which were 3, 24, 48, 72, and 96 hours old.

A modification of the method of Child and Purdy (1926) for making ink imprints was used. The ink mixture was prepared by using one-half mimeograph ink and one-half glycerine. A piece of blotting paper was cut slightly larger than the piece of cake from which the print was to be made, and the paper was placed on a thin piece of soft wood which was in turn somewhat wider and longer than the paper. With a soft brush as much as possible of the ink mixture was worked into the blotting paper. A piece of muslin was then drawn tightly over it and was held securely in place by thumb tacks. Finally, more ink was added. Excess ink on the surface was carefully avoided for this was found to result in an uneven print, wanting in clearness.

The cake was placed on the blotter and pressed lightly by using a small board to insure evenly distributed pressure and a satisfactory imprint of the entire surface. The cake was transferred to newsprint on which the print was to be made. The sample was again pressed lightly with the board, making sure that every portion of the slice touched the paper. Quickly blotting the imprint with a clean blotter made a clearer print as the ink had no tendency to blur. After making each print the blotting surface was cleaned by lightly scraping the surface with a case knife. New ink was added as necessary but care was taken never to have an excess.

The ink imprints were examined by three members of the foods staff of the Department of Home Economics. One out of the set of five series was chosen as representative, and this set was photographed.

RESULTS AND DISCUSSION

The compressibility readings of the entire study ranged from approximately 65 to 85 grams (Table 1). It was noted that the compressibility

readings for the fresh cakes were approximately 65 grams, with a gradual increase throughout the storage period, until after 96 hours when compressibility readings of between 80 and 85 grams were obtained.

TABLE 1

Effect of Kind of Flour on Compressibility of Cake

Kind of flour	Mean compressibility and standard deviation of the mean (16 samples in all cases)	Difference between means and standard error of difference in compressibility according to type of flour			
		Cake flour as basis for comparison	High-fat soy flour as basis for comparison	Low-fat soy flour as basis for comparison	Minimum-fat soy flour as basis for comparison
A. 3 HOURS AFTER BAKING [1]					
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Cake	67.2±0.9	....	2.3±1.4	0.9±1.3	0.6±1.5
High-fat soy	64.9±1.0	2.3±1.4	....	3.2±1.3	1.7±1.5
Low-fat soy	68.1±0.9	0.9±1.3	3.2±1.3	....	1.5±1.4
Minimum-fat soy	66.6±1.1	0.6±1.5	1.7±1.5	1.5±1.4	....
B. 24 HOURS AFTER BAKING [1]					
Cake	76.2±1.0	....	2.8±1.4	0.8±1.5	1.1±1.5
High-fat soy	73.4±1.0	2.8±1.4	....	2.0±1.5	1.7±1.4
Low-fat soy	75.4±1.1	0.8±1.5	2.0±1.5	....	0.3±1.5
Minimum-fat soy	75.1±1.0	1.1±1.5	1.7±1.4	0.3±1.5	....
C. 48 HOURS AFTER BAKING [1]					
Cake	79.9±1.1	....	2.6±1.6	0.2±1.7	1.2±1.4
High-fat soy	77.3±1.2	2.6±1.6	....	2.8±1.8	1.4±1.5
Low-fat soy	80.1±1.3	0.2±1.7	2.8±1.8	....	1.4±1.6
Minimum-fat soy	78.7±1.0	1.2±1.4	1.4±1.5	1.4±1.6	....
D. 96 HOURS AFTER BAKING [1]					
Cake	84.9±1.1	....	2.7±1.4	0.0±1.9	1.2±1.8
High-fat soy	82.2±0.9	2.7±1.4	....	2.7±1.8	1.5±1.7
Low-fat soy	84.9±1.5	0.0±1.9	2.7±1.8	....	1.2±2.1
Minimum-fat soy	83.7±1.4	1.2±1.8	1.5±1.7	1.2±2.1	....

[1]Approximately; in no case was the difference significant.

The particular interest in compressibility tests has been to determine whether there is a significant difference (three or more times the standard error)[4] between the standard cake and any of the soy-flour cakes at different periods after baking.

Comparing the results on this basis, it was noted that the differences in compressibility of the standard product and the three different types of soy-flour cakes between three and 24 hours were statistically significant.

[4]Snedecor, George W., *Statistical Methods*, 1937.

Standard deviation of the mean:

$$s\bar{x} = \sqrt{\frac{\Sigma x^2}{N(N-1)}}$$

Standard error of the difference:

$$\sqrt{(s\bar{x}_1)^2 + (s\bar{x}_2)^2}$$

However, in no case was the difference between 24 and 48 hours or between 48 and 96 hours significant.

Using this same basis of comparison the effect of the kind of flour on compressibility of the cakes was considered. The data all showed, according to the type of flour, no significant differences in 3, 24, 48, or 96 hours after baking.

According to the results of subjective ratings for freshness (Table 2) all of the cakes staled to such an extent from one period to another that there were statistically significant differences between periods for each type of flour. The ratings for general desirability are not presented in this paper, but the amount of change in this quality from one period to another followed the same pattern as for freshness with one exception, namely, that the low-fat soy flour cake showed no significant difference between three and 24 hours of storage.

TABLE 2

Effect of Kind of Flour on Freshness of Cake as Rated Subjectively

A. 3 HOURS AFTER BAKING*[1]

Kind of flour	Mean score and standard deviation of mean [2]	Difference between means and standard error of difference in subjective rating according to type of flour			
		Cake flour as basis for comparison	High-fat soy flour as basis for comparison	Low-fat soy flour as basis for comparison	Minimum-fat soy flour as basis for comparison
Cake	4.99±.01	....	.01±.02	.00±.02	.02±.10
High-fat soy	4.98±.01	.01±.02	....	.01±.02	.01±.04
Low-fat soy	4.99±.01	.00±.02	.01±.02	....	.02±.04
Minimum-fat soy	4.97±.03	.02±.10	.01±.04	.02±.04	....

B. 24 HOURS AFTER BAKING*[3]

Cake	3.88±.05	....	.12±.09	.18±.10	.43±.10
High-fat soy	3.76±.07	.12±.09	....	.06±.12	.31±.11
Low-fat soy	3.70±.09	.18±.10	.06±.12	....	.25±.13
Minimum-fat soy	3.45±.09	.43±.10	.31±.11	.25±.13	....

C. 72 HOURS AFTER BAKING*[4]

Cake	2.26±.73	....	.12±.01	.11±.14	.37±.01
High-fat soy	2.38±.74	.12±.10	....	.23±.14	.49±.10
Low-fat soy	2.15±.12	.11±.14	.23±.14	....	.26±.14
Minimum-fat soy	1.89±.69	.37±.01	.49±.10	.26±.14	....

* Approximately.   [1] None of the differences is significant.   [2] Out of a possible score of five.   [3] The cake-flour product is significantly better than the minimum-fat soy flour product.   [4] The cake-flour and the high-fat soy flour products are significantly better than the minimum-fat soy flour product.

In a comparison of the staling for the three types of cake it was found that at three hours after baking all the cakes had about the same freshness rating. At the end of 24 hours the cake-flour product was rated significantly fresher than the minimum-fat soy flour cake. Even though the mean scores of the high-fat and low-fat soy flour cakes were lower than the mean score of the cake-flour product, the differences were not statistically significant. Thus, in all possible comparisons the only significant difference was that found between the cake-flour product and the minimum-fat soy flour product.

By 72 hours after baking, the cakes made from cake flour and high-fat soy flour, respectively, were both significantly fresher than the cakes made from minimum-fat soy flour. Here again, the low-fat soy flour cakes were not significantly staler than the cake-flour product though they were not significantly fresher than the minimum-fat cakes.

Differences in general desirability ratings, according to the type of flour used, showed the standard cake to be significantly more desirable than any of the soy-flour cakes at the end of three and also 24 hours after baking; however, at the end of 72 hours the cake-flour products were no longer significantly more desirable than the low-fat soy flour cakes and both the cake-flour and the high-fat soy flour cakes were significantly more desirable than the minimum-fat soy flour cakes.

After noting differences in the general desirability and freshness ratings on the score cards it was not considered necessary to make a statistical analysis of the texture, moistness, tenderness, grain, color, flavor, and degree of acceptability ratings. It seemed advisable, however, to give the judges an opportunity to supplement their score-card records by comments on general impressions gained concerning the effect of soy flour in fresh and stored cakes. In these comments the judges reported that the texture and grain of the soy-flour cakes when fresh were not as smooth and velvety as in the standard product. When fresh, no difference was evident between the moistness of the standard cake and of the soy-flour cakes. Little difference was apparent between the tenderness of the standard and that of the soy-flour products. The color of the soy-flour cakes, though somewhat darker than that of the standard, was not dark enough, as a rule, to be objectionable. The flavor of soya was fairly distinct and, while not always undesirable, was not considered altogether pleasing.

When the stored cakes were compared in a similar fashion, it was indicated that soy flour caused no improvement in texture, and varied comments with regard to moistness were made. Some judges felt that the soy-flour products seemed to retain moisture better than the standard cakes, but others reported little difference on this basis. The general opinion was that after five days, the soy-flour cakes and the standard were similar in tenderness. The grain of the soy-flour cakes, on the whole, seemed to become rather coarse in comparison with that of the standard. The color of some soy-flour cakes deepened more than the color of the standard ones, but considerable difference was evident between different soy-flour products. The soy-flour flavor became particularly evident in some products, so much so as to make them rather objectionable, but in others the flavor was quite pleasant.

Most of the judges felt that the results of the use of soy flour in cake were rather disappointing. Two judges felt that the soy-flour cakes which they considered the best retained a "richness" of quality which the plain cake on storage for several days did not possess. However, they were not able to indicate the particular characteristic contributing to the quality they referred to as "richness." In general, the judges felt there did not seem to be any consistent effect of soy flour on the staling process.

A study of the ink imprints of the different cakes at various storage periods (Fig. 1) revealed some interesting features regarding the grain of

the cakes. On the whole, the soy-flour cakes were very similar to the standard ones when compared in three hours and also 24 hours after baking. At the end of 48 hours all of the cakes, including the standard, were slightly coarser than at 24 hours. After 72 hours a change in grain was very evident. The cakes were compact in part and coarse in part, resulting in a very uneven texture. After observing the ink imprints the judges were of the opinion that the minimum-fat soy flour cakes became coarser than the other cakes between 48 and 72 hours. The low-fat soy flour cakes appeared very compact 72 hours after baking, and at the end of 96 hours they had become quite coarse. After 96 hours the soy-flour cakes, in general, were coarser than the standard products.

In the opinion of the judges the ink imprints as reproduced (Fig. 1) give a fairly satisfactory picture of the grain of the products as they actually appeared. This set is typical of all five sets examined by the judges.

CONCLUSIONS

Compressibility readings for fresh cakes were approximately 65 grams with a gradual increase throughout the storage period of 96 hours, at which time readings of between 80 and 85 grams were obtained.

The greatest change in compressibility readings of the cakes took place between three and 24 hours after baking. The kind of flour used had no differential effect on compressibility of the cakes 3, 24, 48, or 96 hours after baking.

Evidence that the method of testing compressibility gave reproducible data is found in the fact that the figure for the between-the-average readings of any two of the five duplicate cakes ranged from ±0.6 to ±2.0 grams.

All of the cakes staled to such an extent from one test period to another that the differences in the subjective ratings for the different periods were statistically significant for each type of flour when compared with its rating for a previous or succeeding period. In general, the amount of change in general desirability followed the same pattern as for change in freshness, as rated by the subjective ratings.

Three hours after baking all of the cakes were rated subjectively as having about the same degree of freshness. By 72 hours after baking, according to the subjective ratings, the cakes made from cake flour and those made from high-fat soy flour were both significantly fresher than the cakes made from the minimum-fat soy flour. However, the low-fat soy flour cakes were not significantly staler than the cake-flour product nor fresher than the minimum-fat soy flour cakes.

In general, the judges felt there did not seem to be any consistent effect of soy flours on the staling process of cakes. The difference in the quality of cakes made from different soy flours made it difficult for the judges to give impressions between the soy-flour cakes as a group and the standard cakes. Most of the judges felt that the use of soy flour gave rather disappointing results.

A study of the ink imprints of the different cakes at various storage periods revealed some interesting features regarding the grain of the cakes. On the whole, the soy-flour cakes were very similar in grain to the standard cakes when compared three and 24 hours after baking. The
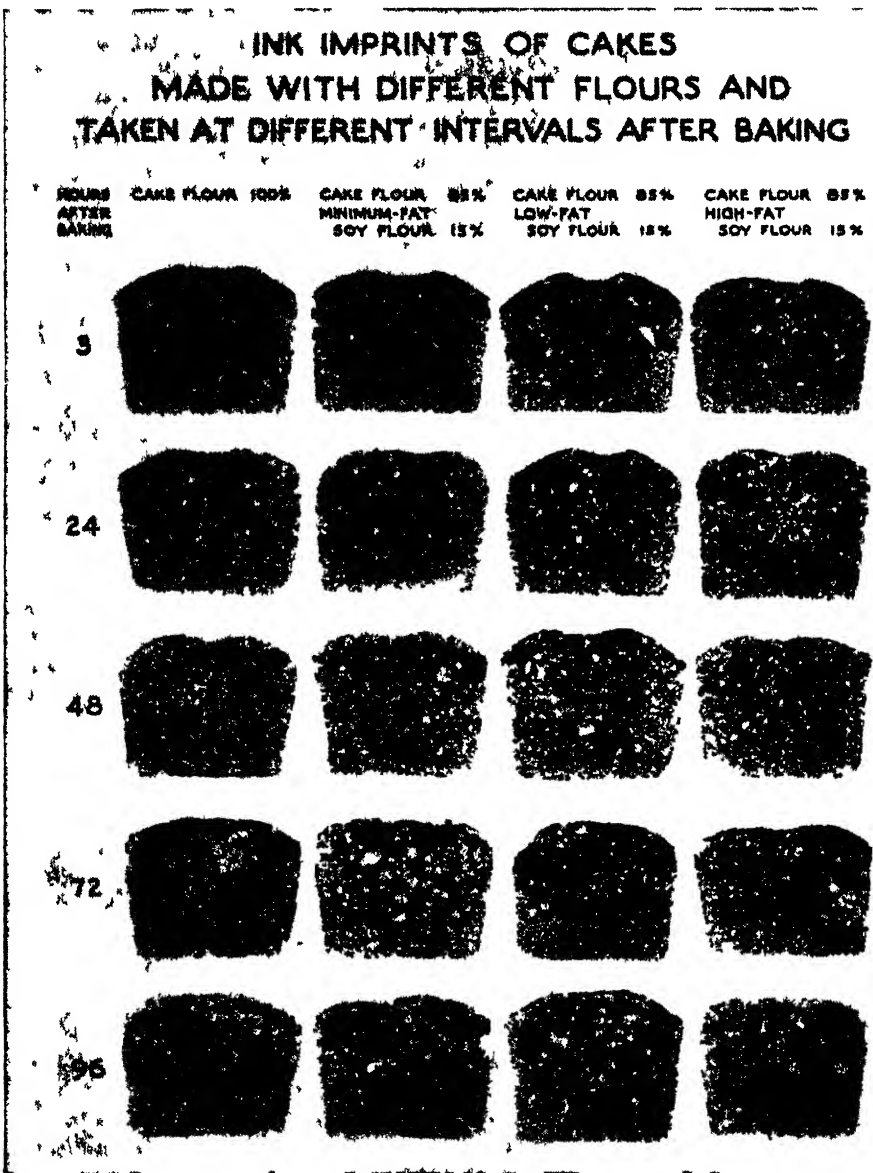
FIG 1

minimum-fat soy flour cakes became coarse more rapidly than the other cakes. After 96 hours the soy-flour cakes, in general, were coarser than the standard cakes.

SUMMARY

It was considered desirable to undertake a study of the value of different types of soy flour in retarding staling of cakes. Representative samples of low-, minimum-, and high-fat soy flours were chosen for study. By weight, approximately 15 per cent of the total flour in the cakes was soy flour and the remainder cake flour.

Each time the cakes were prepared one formula (seven cakes) was made from cake flour and from each of the three types of soy flour. After the cakes were cooled and wrapped in waxed paper they were stored in cans with tightly fitting lids. After definite storage periods cakes were judged by several tests.

Of the various methods generally available at present for measuring the rate of staling, compressibility tests, ink imprints, and subjective judging of the cakes have been included in this investigation.

For use in this laboratory, a new type of compressibility apparatus was designed and constructed. Compressibility readings as affected by each type of flour were obtained from a total of 16 batches of cakes. A statistical analysis was made of the compressibility readings to determine which results showed significant differences.

The three cakes (from each batch of seven) not used for compressibility determinations were used for subjective judging on the day they were baked and when approximately 24 and 72 hours old. A statistical analysis was made of the freshness and general desirability ratings to determine which ratings showed significant differences.

Five batches of the four types of cake were baked especially for the purpose of making ink imprints. One cake from each batch was placed in a freezing unit when 3, 24, 48, 72, and 96 hours old. After 24 hours in the freezing unit the cake was removed and ink imprints were recorded immediately. The complete set of ink imprints (five series) was examined; one series out of the set of five was chosen as representative of the set and this series was photographed.

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EFFECTIVENESS OF FAT IN SOY FLOUR AS A SHORTENING AGENT [1]

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The fat content of the soy flours being manufactured at present varies from less than one to more than 20 per cent, and the question has been raised as to whether the fat in the high-fat soy flours is effective as a shortening agent in baked products. Some processors have stated that the fat does not serve effectively as a shortening agent, and they have recommended that it be removed from the flour and used more profitably for other purposes. However, the value of the fat in the soy flour when it is used in batters and doughs needs to be investigated before such statements can be accepted. This study was undertaken in order to determine whether the fat which is present in soy flours functions as a shortening agent in pastry.

REVIEW OF LITERATURE

Soy flours are usually classified according to their fat content as (1) full-fat soy flour, containing all of the fat of the soybean, (2) low-fat soy flour, containing four to eight per cent fat, and (3) minimum-fat soy flour, containing less than one per cent fat.

From the standpoint of baking quality the chief interest in the fat of the soy flour is with regard to its effectiveness as a shortening agent. Workers are not in agreement as to its value for this purpose. In personal communications with the authors, several workers have commented on this subject. One such worker has stated that the fat in full-fat soy flour is not effective as a shortening agent. In another communication, the opinion has been expressed that the fat in full-fat soy flour has an appreciable shortening action. Since the shortening effect was greater than can be accounted for by the fat in the flour, it was suggested that the lecithin present increased the shortening action by promoting a more even distribution of the fat. According to Tremple (1944), approximately 75 per cent of the oil of the full-fat soy flour is available for shortening; and when this type of soy flour is used, the amount of shortening in the formula may be reduced by an amount equal to one-sixth of the weight of the soy flour. Gabel and Sunderlin (1945) found that the fat in soy flours containing 5, 15, and 22 per cent fat exerted a shortening action in pastry made from soy flour, fat, and water. They reported that the fat in two extracted soy flours containing five and 15 per cent fat had approximately the same shortening value as equivalent amounts of plastic fats.

Also in a personal communication, one worker states that some of the fat in the full-fat soy flour is tied up in the cells of the flour and may not be available for shortening purposes. This statement suggests that a microscopic examination of pastry dough made from a blend of wheat and soy flour might show a characteristic manner of fat distribution in the dough. Microscopic examinations of batters and doughs reported to date have apparently been confined to problems concerning cakes and breads; methods for microscopic studies of pastry dough need to be developed.

EXPERIMENTAL PROCEDURE

Pastry wafers prepared from wheat flour and from blends of 80 per cent wheat flour and 20 per cent soy flour were used to determine the effect of the type of soy flour on the breaking strength of the wafers. The soy flours used in this investigation were obtained from one processor. This particular processor manufactures four types of soy flour and refers to them as full-fat, high-fat, low-fat, and minimum-fat soy flours.

The fat content of the wheat flour and of each of the soy flours used in preparing the wafers was determined by the official A.O.A.C. (1940) direct method for the crude fat or ether extract of cereals. The results reported are averages of three determinations.

The wafers for the breaking-strength study were prepared according to the following formulae:

Ingredient	Wheat-flour pastry	Wheat- and soy-flour pastry
	gm.	*gm.*
Wheat flour	307.0	245.6
Soy flour	....	61.4
Hydrogenated fat	133.2	133.2
Water	105.0	105.0
Gluten	10.0	10.0
Salt	6.0	6.0

In the preliminary tests wafers containing 20 per cent of the flour by weight as soy flour had average breaking strengths of seven to eight ounces. It was felt that higher breaking strength values would allow a greater range in the breaking strengths of the wafers made from the different flours. Dry gluten was prepared in the laboratory by the method of Aitken and Geddes (1938) and added to the dry ingredients used in the doughs. The breaking strengths of the wafers were significantly increased thereby in all cases.

In preparing the wafers the ingredients were combined as follows: The fat was mixed for two minutes at low speed in a Kitchen-Aid mixer. The machine was stopped, the sides of the bowl were scraped down with a rubber scraper, and the mixing was continued for three minutes longer. During the creaming of the fat the dry ingredients were sifted once and "tabled," as described by Fales (1925) for the sampling of powdered materials for quantitative analysis. The mixer was stopped and the contents of the bowl were scraped down. Approximately one-third of the dry ingredients was added to the fat in the bowl, and 35 grams of water were

placed in an oil dropper attached to the mixer. The dry ingredients and the fat were mixed at low speed for 40 seconds, the water being added gradually during the first 30 seconds of mixing. The contents were again scraped down, the remainder of the dry ingredients added, and mixed at low speed for 50 seconds. Seventy grams of water were added from the oil dropper during the first 40 seconds of final mixing.

The dough was shaped into a ball, wrapped in wax paper, and stored in a refrigerator for two hours. It was divided into four parts for rolling and was hand-rolled between wax paper to a thickness of one-eighth inch as regulated by pastry gauges. It was then rolled between wax paper through a hand-model clothes wringer, using metal gauges one-sixteenth inch thick to control the thickness. It was cut into wafers three and one-fourth by one and five-eighths inches. The wafers were pricked to prevent blistering, and baked on an aluminum baking sheet in a gas oven at 204.4°C.(400°F.) for nine minutes. They were cooled on wire cake racks for two hours, and the breaking strengths were then measured on the Bailey shortometer.

Each type of pastry was prepared 11 times, and from 16 to 20 wafers were prepared from each batch of dough. In all, the breaking strengths of approximately 200 wafers of each type of pastry were determined.

Microscopic sections of raw pastry dough were prepared by the following method: Raw samples of pastry approximately one millimeter square and one and one-half millimeters thick were embedded in 10 per cent gelatin, incubated on a warming plate at 45°C.(113°F.) for two hours, and placed in the refrigerator overnight. A gelatin block containing a pastry sample was cut from the bed, and frozen sections 15 microns thick were prepared with the sliding microtome. The sections were stained in Sudan IV solution for five minutes and then mounted in Kaiser's glycerin jelly. The staining solution and the glycerin jelly were made according to Krajian's (1940) directions. Examination of the sections under the microscope at a magnification of 150 $\times$ revealed that the fat was stained an intense red color. Photomicrographs of a few sections were prepared at this magnification. The portion stained with Sudan IV appears black in the finished photomicrograph.

RESULTS AND DISCUSSION

The average figures for the fat content of the full-fat, high-fat, low-fat, and minimum-fat soy flours were 21.55, 12.06, 6.21, and 0.57 per cent, respectively. The fat content of the wheat flour was 0.69 per cent, a value slightly higher than that for the minimum-fat soy flour. These values were used to calculate the amount of total fat in each type of pastry dough prepared in the breaking-strength study. The amount of total fat was the sum of the weight of the hydrogenated fat and the fat in the wheat and soy flour; the calculated amount of total fat in each of the doughs is shown (Table 1).

The results of the breaking-strength study are also reported (Table 1). The breaking strengths of the wafers made from wheat flour and from each of the blends of wheat and soy flour were analyzed statistically in order to determine if the differences in breaking strength were significant.

TABLE 1

Breaking Strengths of Wafers Prepared From Wheat Flour and Blends of Wheat and Soy Flours

Flour used in pastry wafers	Total fat in dough	Number of samples	Mean breaking strength of wafers and standard deviation of mean[1]	Difference between mean breaking strengths and standard error of difference[3]				
				Wheat flour as a basis of comparison	Minimum-fat soy flour as a basis of comparison	Low-fat soy flour as a basis of comparison	High-fat soy flour as a basis of comparison	Full-fat soy flour as a basis of comparison
	gm.		*oz.*	*oz.*	*oz.*	*oz.*	*oz.*	*oz.*
Wheat.................	135.32	206	12.5±.23	....	1.5³±.29	1.3³±.31	2.8³±.33	3.0³±.29
Wheat and minimum-fat soy flour........	135.24	207	11.0±.19	1.5³±.29	....	0.2±.28	1.3³±.31	1.5³±.26
Wheat and low-fat soy flour........	138.70	211	11.2±.21	1.3⁰±.31	0.2±.28	....	1.5³±.32	1.7³±.27
Wheat and high-fat soy flour........	142.29	201	9.7±.24	2.8³±.33	1.3³±.31	1.5³±.32	....	0.2±.30
Wheat and full-fat soy flour........	148.12	213	9.5±.18	3.0³±.29	1.5³±.26	1.7³±.27	0.2±.30	....

[1] Standard deviation of the mean, Sherman (1937), $= S\bar{x} = \sqrt{\dfrac{\Sigma x^2}{n\,(n-1)}}$ in which $S\bar{x}$ = standard deviation of the mean, x = deviations of the individual values from the mean, n = total number of values for the variable. [2] Standard error of the difference between two means, Nason (1939), $= \sqrt{(S\bar{x}_1)^2 + (S\bar{x}_2)^2}$.
[3] Difference is considered significant.

The mean breaking strength of the wheat-flour wafers was significantly higher than the breaking strength of wafers made from each blend of wheat and soy flour in turn. This indicated that substitution of soy flour for 20 per cent of the wheat flour in the pastry formula lowered significantly the breaking strength of the wafers. Bohn and Favor (1944) have reported that a blend of small amounts (three per cent) of full-fat soy flour with wheat flour did not change the character of the Farinograph curve, but that higher amounts (20 per cent) showed a decrease in the strength of the wheat- and soy-flour blend.

The mean breaking strengths of the wafers made from the blends of wheat and soy flours were analyzed statistically in order to determine whether the type of soy flour caused a significant difference in the breaking strength of the wafers. The mean breaking strengths of the wafers made from the wheat and full-fat soy flour blend and those from the wheat and high-fat soy flour blend were both significantly lower than the mean breaking strengths of the wafers made from the wheat and low-fat soy flour blend and from the wheat and minimum-fat soy flour blend. On the other hand, the difference between the mean breaking strengths of the wafers made from the wheat and low-fat soy flour blend and the wheat and minimum-fat soy flour blend was not significant, nor was the difference between the mean breaking strength of the wafers made from the wheat and full-fat soy flour blend and that of those made from the wheat and high-fat soy flour blend significant.

These differences in breaking strength indicate that the use of full-fat and high-fat soy flour in pastry increased the shortness of the wafers. The differences in the fat content of the soy flours resulted in differences in the amount of total fat in the pastry doughs prepared from the blends of wheat and soy flour and was probably responsible for the differences in the mean breaking strengths of the wafers. However, other variations in the composition of the soy flours may also have affected the breaking strengths of the wafers.

The chief contribution of the microscopic part of this investigation has been the development of a satisfactory method of preparing sections of raw pastry dough stained to show fat. A limited study was made of the distribution of the fat in each of the pastry doughs prepared in the breaking strength study but relatively few sections were prepared. Examination of the sections failed to show definitely a characteristic distribution of the fat in any type of dough. Examination of a large number of sections prepared from different parts of the dough and from many experimental batches of dough would be necessary before conclusions could be drawn as to the manner of the fat distribution in the different doughs.

Photomicrographs of the sections of raw pastry dough are presented (Fig. 1) to illustrate the results which were obtained by the histological method which was developed. (For reproduction in this paper the photomicrographs were reduced to approximately one-third of their original size, hence the resulting magnification is approximately 50 times.) An effort was made to select for photography fields which were considered typical for each of the doughs. It should be stated, however, that the appearance

of the fields varied widely among the different sections prepared from each of the doughs.

SUMMARY AND CONCLUSIONS

Pastry wafers were prepared from wheat flour and from blends of wheat flour and each of four types of soy flour in turn: full-fat, high-fat,
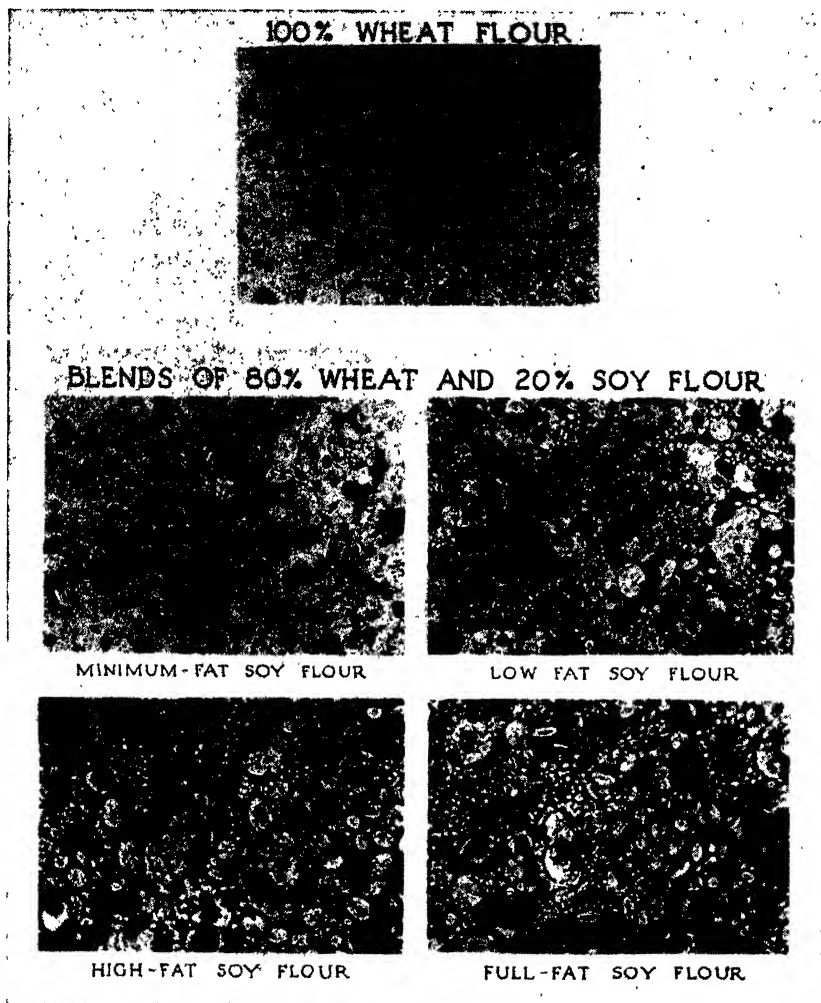
FIG. 1. Pastry doughs prepared from wheat and soy flours. Fat stained with Sudan IV. Magnification × 50.

low-fat, and minimum-fat soy flour. The breaking strengths of these wafers were measured by the Bailey shortometer and used to determine the effect of the type of soy flour on the breaking strength of the wafers. The fat content of the soy flours and of the wheat flour was determined, and the amount of total fat in each of the doughs used in preparing the wafers was calculated.

The resulting breaking strengths of the wafers from each type of flour were analyzed statistically to determine whether the difference in the mean breaking strengths were significant. The mean breaking strength of the wafers made from wheat flour was significantly higher than the values for the wafers made from each blend of wheat and soy flour in turn. The mean breaking strengths of the wafers made from blends of wheat and full-fat soy flour and from wheat and high-fat soy flour were significantly lower than the mean breaking strengths of wafers made from the wheat and low-fat soy flour blend and the wheat and minimum-fat soy flour blend. The differences in the mean breaking strengths of the wafers made from the wheat- and soy-flour blends indicated that the use of full-fat and high-fat soy flour in pastry increased the shortness of the wafers.

A satisfactory method was developed for the preparation of microscopic sections of raw pastry dough. Frozen sections 15 microns thick were prepared and stained with Sudan IV to show fat, and photomicrographs of some of these were prepared. The limited number of microscopic sections included has not warranted definite conclusions concerning the manner of the fat distribution in the pastry dough made from wheat flour and from blends of wheat and soy flours.

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THIAMIN AND ASCORBIC ACID VALUES OF RAW
AND CANNED PEAS

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Except for the data collected in the current nutrition program sponsored by the National Canners Association and the Can Manufacturers Institute outlined by Pilcher and Clark (1946), Clifcorn (1946), and Esty (1946), little recent information on the vitamin contents of commercially canned foods has appeared in the literature since the pioneer studies of Kohman and Eddy and their associates (1937).

The data presented and discussed in this report represent a survey carried out prior to the N. C. A.- C. M. I. program on the thiamin and ascorbic acid values of market samples of canned peas which make up a considerable portion of the annual canned vegetable production. Samples were obtained which were representative of the production of all of the important pea-canning sections of the country. Also shown are a number of values for the thiamin and ascorbic acid contents of raw peas grown in one of the major pea-canning areas. The same samples were also analyzed for calcium, magnesium, and the proximate components; the data obtained were the subject of an earlier paper by Alexander, Sallee, and Taylor (1943).

The investigation was limited to the study of thiamin and ascorbic acid for several reasons. First, the determinations of both ascorbic acid and thiamin by chemical methods are relatively rapid and may readily be adapted to field work. Second, the two nutrients studied are probably the most labile of the better known factors; and their water solubility, the susceptibility of ascorbic acid to oxidation, and the sensitivity of thiamin to heat make a study of these two a particularly good index of the probable over-all vitamin retention. Third, peas are a reasonably good source of both thiamin and ascorbic acid, consequently the detection of significant variations is well within the precision of the methods employed.

THIAMIN AND ASCORBIC ACID IN RAW PEAS

This study of the thiamin and ascorbic acid values of raw peas was carried out in order to obtain data which would reveal the extent to which variations of these values in the raw stock might be reflected in the concentrations of these two nutrients in the canned product. The samples were obtained from one of the major pea-canning sections of Wisconsin and were analyzed in a field laboratory to avoid the difficulties associated with the transportation of fresh samples. Samples of the Alaska or early, smooth-skinned peas and of several varieties of the sweet or wrinkled types were analyzed. The survey was repeated during each of two consecutive seasons.

Sampling: A sample of approximately 50 pounds of freshly vined peas was separated by hand sieving into as many sieve-size fractions as possible.

Each size grade was washed in cold water and the broken peas removed. After washing and draining, representative samples were removed for analysis. Each analysis was carried out in duplicate.

Analysis: The nature of the studies reported, as well as the scope of the project undertaken, made it necessary to use chemical methods for the estimation of the vitamins. All analyses for thiamin were made using a thiochrome method based on that of Hennessey (1941). Ascorbic acid was determined using a titrimetric method modified from that of Bessey and King (1933).

TABLE 1

Average Thiamin and Ascorbic Acid Contents of Raw Peas

Variety	Sieve size	Thiamin [1]		Ascorbic acid [1]	
		1941	1942	1941	1942
		mg./100 gm.	mg./100 gm.	mg./100 gm.	mg./100 gm.
Alaska	1	.24	....	39	....
	2	.28	.27	32	60
	3	.31	.35	27	50
	4	.34	.36	27	46
Pride	2	.37	.28	31	64
	3	.42	.33	29	66
	4	.41	.34	23	48
	5	.46	.39	21	46
	6	.47	.39	22	43
	7	....	.41	....	38
Profusion	3	.30	....	21	....
	4	.31	.35	17	22
	5	.33	.35	16	20
	6	.33	.39	17	16
	7	.37	.44	16	16
Perfection	1	.34	....	33	....
	2	.34	.28	28	33
	3	.39	.29	27	32
	4	.49	.30	25	33
	5	.52	.34	21	30
	6	.57	.40	20	26
Pennin	4	....	.40	....	25
	5	....	.45	....	21
	6	....	.50	....	17
	7	....	.53	....	17
Alderman	4	.38	....	28	....
	5	.40	....	25	....
	6	.45	....	23	....
	7	.45	....	21	....

[1]Average of duplicate determinations.

Discussion: Results of the analyses of raw peas (Table 1) show that the most significant variable is that of sieve size. The thiamin values increase with increasing sieve size and the ascorbic acid values decrease. There were no marked differences between varieties and no significant

differences which might be attributed to seasonal variations. The data demonstrate, however, that variations in the raw canning stock are great enough to account for the wide ranges actually observed in the thiamin and ascorbic acid values of the canned products. The values for ascorbic acid in the 1942 samples of Alaska and Pride varieties are significantly higher than those found for comparable samples in 1941. This variation in content of nutrients in raw canning stocks has been repeatedly noted by workers in this field. The higher values obtained suggest that an over-all increase in the vitamin contents of raw vegetables through improved plant breeding, selection, and cultural methods might be affected.

THIAMIN AND ASCORBIC ACID VALUES OF CANNED PEAS

The primary object of the survey of canned peas was to establish the ranges for thiamin and ascorbic acid contents of canned peas as they reach the consumer.

TABLE 2

Food Inspection Data on Canned-Pea Samples

Can size	Number of cans examined	Vacuum (mercury)		Headspace		Net weight		Drained weight	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
		in.	in.	in.	in.	oz.	oz.	oz.	oz.
303 x 406	41	0	16	7/32	11/32	16.9	17.5	9.8	11.8
307 x 409	66	0	13	6/32	16/32	20.2	21.7	12.1	15.0
404 x 700	7	0	12	9/32	17/32	50.9	53.1	32.3	34.1
603 x 700	15	0	11	7/32	17/32	110.8	114.3	69.9	79.4

Sampling: Samples were chosen which represented all of the major pea-canning districts of the country. Peas of different varieties, sieve sizes, and quality grades packed in containers of the more commonly used sizes were included. The survey included samples from two consecutive packing seasons. The major portion of the samples was of the conventionally canned type, but also included were a number of samples canned by a relatively new pea-canning procedure—Blair (1940) and Blair and Ayres (1943). This procedure, known in the trade as the "Blair" process, consists essentially of the use of mild alkaline reagents to hold the peas at a slightly elevated pH level during the process. A second essential part of the procedure is the application of a high temperature in the thermal sterilization. The duration of the heat treatment is materially shortened yet still produces the same sterilizing effect. The combination of these two points of departure results in a marked increase in the retention of the natural chlorophyll content of the raw peas. In order to obtain samples with known histories, case lots were obtained either directly from canners' stocks or from wholesalers' warehouses.

Analysis: At least two cans of each lot were opened for analysis and the vacuum headspace and fill were recorded at the time of opening. These values all fell within normal commercial limits as shown by the data (Table 2). The brine and drained peas were weighed and analyzed separately, all analyses being carried out in duplicate.

TABLE 3

Thiamin and Ascorbic Acid in Commercially Canned Peas

Type of process	Variety	Sieve size and quality	Can size	Year packed	Locale	Number cans analyzed	Average thiamin content	Average ascorbic acid content
							mg./ 100 gm.	mg./ 100 gm.
Conventional	Alaska	Fancy No.3	307x409	1940	Central	3	.13	13
Conventional	Alaska	Fancy No.3	303x406	1940	Central	2	.09	12
Conventional	Alaska	Fancy No.3	307x409	1940	Atlantic	3	.08	10
Conventional	Alaska	Fancy No.3	307x409	1941	Central	2	.09	8
Conventional	Alaska	Fancy No.3	303x406	1941	Central	3	.10	12
Conventional	Alaska	Fancy No.3	307x409	1941	Atlantic	2	.09	9
Conventional	Alaska	Extra Std. No.3	603x700	1940	Central	2	.09	10
Conventional	Alaska	Extra Std. No.3	603x700	1941	Central	3	.09	12
Conventional	Alaska	Standard No.4	603x700	1940	Central	2	.11	11
Conventional	Alaska	Standard No.4	307x409	1940	Central	3	.09	10
Conventional	Alaska	Standard No.4	307x409	1940	Atlantic	3	.09	9
Conventional	Alaska	Standard No.4	307x409	1941	Central	2	.12	9
Conventional	Alaska	Standard No.4	603x700	1941	Central	3	.09	11
Conventional	Alaska	Standard No.4	303x406	1941	Central	2	.11	9
Conventional	Alaska	Standard No.4	307x409	1941	Atlantic	2	.07	9
Blair	Alaska	Fancy No.4	303x406	1941	Central	4	.10	9
Blair	Sweet	Fancy No.3	303x406	1940	Central	4	.10	9
Blair	Sweet	Fancy No.3	404x700	1940	Central	3	.11	9
Conventional	Sweet	Fancy No.4	307x409	1940	Central	3	.12	8
Conventional	Sweet	Fancy No.4	603x700	1940	Central	3	.13	12
Conventional	Sweet	Fancy No.4	307x409	1940	Central	3	.12	10
Conventional	Sweet	Fancy No.4	307x409	1940	Pacific	3	.11	11
Blair	Sweet	Fancy No.4	303x406	1940	Central	4	.11	10
Blair	Sweet	Fancy No.4	303x406	1940	Pacific	4	.11	13
Conventional	Sweet	Fancy No.4	307x409	1941	Central	2	.12	9
Conventional	Sweet	Fancy No.4	307x409	1941	Central	3	.13	11
Conventional	Sweet	Fancy No.4	307x409	1941	Atlantic	3	.15	8
Conventional	Sweet	Fancy No.4	307x409	1941	Atlantic	2	.13	8
Conventional	Sweet	Fancy No.4	307x409	1941	Atlantic	2	.11	8
Conventional	Sweet	Fancy No.4	307x409	1941	Pacific	2	.11	13
Blair	Sweet	Fancy mixed	303x406	1940	Central	3	.12	8
Blair	Sweet	Fancy mixed	303x406	1940	Central	3	.11	7
Blair	Sweet	Fancy mixed	303x406	1941	Central	4	.10	10
Blair	Sweet	Fancy mixed	303x406	1941	Central	4	.13	10
Blair	Sweet	Fancy mixed	303x406	1941	Central	4	.13	13
Blair	Sweet	Fancy mixed	404x700	1941	Central	4	.10	11
Conventional	Sweet	Standard No.5	307x409	1940	Central	3	.11	8
Conventional	Sweet	Standard No.5	603x700	1940	Central	2	.07	6
Conventional	Sweet	Standard No.5	307x409	1940	Central	3	.12	8
Conventional	Sweet	Standard No.5	307x409	1941	Central	3	.10	8
Conventional	Sweet	Standard No.5	307x409	1941	Central	2	.13	7
Conventional	Sweet	Standard No.5	307x409	1941	Atlantic	3	.12	8
Conventional	Sweet	Standard No.5	307x409	1941	Atlantic	4	.08	12
Conventional	Sweet	Standard No.5	307x409	1941	Pacific	3	.11	6
Conventional	Sweet	Standard No.5	307x409	1941	Atlantic	2	.11	7

Discussion: Results of these analyses are shown (Table 3); the can-size designation, shown in both Table 2 and Table 3, is the can manufacturer's designation and refers to the nominal can dimensions. The first number refers to the diameter, the second to the height; the 307x409 can, commonly known as the No. 2, is nominally $3\frac{7}{16}$ inches in diameter and $4\frac{9}{16}$ inches high. The geographic locations shown as Atlantic, Central, and Pacific include the northeastern states in the Atlantic division; the states of Minnesota, Wisconsin, Illinois, and Indiana in the Central division; and the northwestern states in the Pacific division, including some areas where irrigation is commonly employed.

The analyses of solids and brine were made separately and the values reported have been calculated to show the vitamin values based on the entire contents of the can. In accord with the findings of Brush, Hinman, and Halliday (1944, 1945), it was found that the concentrations of these water-soluble nutrients were approximately the same in both the solid and liquid portions of the container. In general, about one-third of the total amount of either ascorbic acid or thiamin contained in the can was to be found in the brine.

The same wide variation in the thiamin and ascorbic acid contents of canned peas that has been reported by Pressley, Ridder, Smith, and Caldwell (1944) and Ives, Wagner, Elvehjem, and Strong (1944) as a result of work in the N.C.A.- C.M.I. nutrition program, was apparent in the present study. The data also suggest that none of the factors studied show any significant correlation between variety, growing locality, quality grade, or size of container. Differences that do exist are no greater than differences which have been found to exist between raw canning stocks. Canned peas packed by the Blair process were found to fall within the ranges of thiamin and ascorbic acid contents established for conventionally canned peas.

ACKNOWLEDGMENT

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MICROBIOLOGY OF SPRAY-DRIED WHOLE EGG
III. *ESCHERICHIA COLI* [1]

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Between September 1, 1943, and January 1, 1945, 5,410 samples of high-moisture (four to six per cent), spray-dried whole-egg powder manufactured for Lend-Lease shipment were examined for *Escherichia coli*. The samples were obtained from 94 dehydration plants located in 26 states. Plate and direct microscopic count and *Salmonella* studies on samples of this group and on others collected during the same time interval have been reported by McFarlane, Watson, Sutton, and Kurman (1947) and Solowey, McFarlane, Spaulding, and Chemerda (1947).

The test for *E. coli* was selected in preference to the less specific test for the coliform-aerogenes group because *E. coli* is considered a typical fecal species, Parr (1939) and Griffin and Stuart (1940). Its presence in foods and beverages is generally accepted as evidence of fecal contamination. It is in itself an undesirable contaminant, but more significant is the fact that it serves to warn the bacteriologist that other enteric organisms— typhoid, dysentery, and genera responsible for food poisoning and gastroenteritis— may be present. The same portals of entry to the product are available to all of them.

As Hunter (1939) pointed out ". . . there are numerous food products, both simple and compound, in which there is no valid reason for the presence of coliform bacteria under normal conditions. On the other hand, we may recognize the limitations imposed upon an interpretation of the presence of coliform bacteria in products such as garden vegetables, eggs, and dairy products in general." This is a reasonable appraisal. Shell eggs soiled with chicken feces, barnyard manure, humus, and other types of dirt were and still are accepted by the government and by the trade as marketable edible products provided they otherwise conform to the official U. S. standards for quality of individual shell eggs. At the time of this investigation storage eggs, stale current receipts, washed dirty, light dirty, and even some dirty and leaker eggs were among those broken out for dehydration. The best breaking-room technics lessened but did not prevent entry into the liquid egg of bits of fecal material, soiled shell, and other detached debris. Shell-egg washing and sterilizing procedures, when they were practiced, removed the gross dirt but rarely if ever accomplished surface sterilization. There was and still is no satisfactory test for quickly determining the presence of organisms which have already penetrated the shells of eggs unless organoleptically perceptible changes have occurred in the meats.

Our knowledge of the kind of shell eggs being dried, of plant housing conditions, of sanitary practices, and of processing methods prompted our belief that most of the liquid egg produced under commercial conditions in 1943-1944 contained *E. coli*. The question was whether *E. coli* survived the spray-drying process. DeBord (1925) observed ''. . . a big reduction in the total number of viable bacteria, and in some cases a complete loss of the colon-aerogenes group, during the drying process.'' Technical changes have been made in spray-drying processes since DeBord's investigations. Moreover, in order to meet 1943-1944 U. S. Government purchase specifications for egg powder, operators had to use higher grades of raw material (current receipts, storage and frozen eggs) than had been required in 1925 and in the still earlier years of the egg-dehydration industry.

No formal standards for bacterial content were incorporated in the 1943-1944 U. S. Government purchase specifications. The industry was asked, however, to co-operate in manufacturing powder having an *E. coli* count less than 1,000 per gram (absent from 0.001 gram of powder). This unofficial tentative standard corresponded to the 1943 Canadian standard for Grade B powder, Johns (1944). The Canadian standard for Grade A powder required that *E. coli* be absent from 0.01 gram.

The purposes of this investigation were to determine the over-all incidence and degree of *E. coli* contamination in samples of high-moisture, spray-dried whole-egg powder; to determine effects of season, region, and age of samples; and to determine whether any correlation exists between the incidence of *E. coli* and the plate and direct counts, and between the incidence of *E.'coli* and the incidence of *Salmonella*.

EXPERIMENTAL PROCEDURE

E. coli determinations were made according to a modification of the tentative American Public Health Association procedure (1941). The initial 1:10 dilution was prepared by uniformly suspending 11 grams of well-mixed sample in 99 ml. of sterile physiological saline. Serial dilutions were prepared, and measured quantities containing 0.1, 0.01, 0.001, and 0.0001 gram of dried egg were inoculated into lactose broth fermentation tubes. All lactose broth cultures showing gas at the end of 24 hours at 37° C. (98.6° F.) were streaked on Levine's eosin methylene blue agar (Difco). Cultures negative for gas in 24 hours but positive in 48 hours were likewise streaked on EMB agar. After 24 hours' incubation at 37°C., the plates were examined for typical *E. coli* colonies, Levine (1918). Further identification of typical colonies was not made. Suspicious colonies were restreaked on EMB, and isolates were subjected to confirmatory tests—indol, methyl-red, Voges-Proskauer, Koser's sodium citrate, and Eijkman.

RESULTS

E. coli was isolated from 51 per cent of the samples of spray-dried whole-egg powder. It was isolated from the 1:10 dilution (0.1 gram) of powder in 1,266 (23 per cent) samples; from the 1:100 dilution (0.01 gram) in 1,028 (19 per cent) samples; from the 1:1,000 dilution (0.001 gram) in 418 (eight per cent) samples; and from the 1:10,000 dilution (0.0001 gram) in 71 (one per cent) samples. Samples positive in the

TABLE 1

Incidence of E. coli in Egg-Powder Samples, by Plants

Plant No.	Number samples examined	Samples positive in one or more dilutions		Number of samples from which *E. coli* was isolated in dilution of			
				1 : 10	1 : 100	1 : 1000	1 : 10,000
			pct.				
1	69	10	14.5	4	3	3	....
2	141	54	38.3	27	18	8	1
3	57	28	49.1	20	7	1	....
4	44	29	65.9	10	12	6	1
5	79	54	68.4	17	23	11	3
6	225	141	62.7	59	62	17	3
7	52	14	26.9	12	1	1	....
8	53	28	52.8	12	11	4	1
9	60	28	46.7	12	13	3	....
10	54	37	68.5	21	11	5	....
11	89	66	74.2	26	29	10	1
12	65	22	33.8	10	8	3	1
13	528	373	70.6	170	141	45	17
14	121	74	61.2	34	21	17	2
15	62	40	64.5	14	16	9	1
16	82	34	41.5	20	13	1	....
17	171	77	45.0	41	29	7	....
18	121	25	20.7	17	6	2	....
19	73	41	56.2	13	14	11	3
20	64	39	60.9	15	15	9	....
21	176	82	46.6	44	23	14	1
22	153	63	41.2	30	28	4	1
23	99	46	46.5	9	21	13	3
24	209	98	46.9	42	42	14	....
25	213	111	52.1	59	42	9	1
26	59	32	54.2	13	15	4	....
27	160	107	66.9	40	48	16	3
28	118	68	57.6	22	34	9	3
29	79	24	30.4	17	6	1	....
30	106	40	37.7	25	15	....	....
31	58	29	50.0	17	10	2	....
32	94	53	56.4	16	22	10	5
33	44	20	45.5	11	8	1	....
34	73	8	11.0	7	....	1	....
35	43	15	34.9	9	2	4	....
36	47	24	51.1	16	5	2	1
37	166	106	63.9	45	38	22	1
38	48	24	50.0	16	5	3	....
39	37	26	70.3	11	10	5	....
40	63	37	58.7	28	9	....	....
41	68	34	50.0	17	7	10	....
42	87	73	83.9	24	27	20	2
43	78	43	55.1	10	12	14	7

higher dilutions were not always positive in each of the lower dilutions. Approximately 91 per cent of the samples met the War Food Administration (WFA) unofficial tentative standard, that is, *E. coli* was absent in 0.001 gram of egg powder.

Incidence of E. coli in Samples of Egg Powder, by Plants: Ninety of the 94 plants contributed *E. coli*-positive samples. The four plants which did not contribute any positives forwarded very few samples—four, 16, three, and two, respectively. *E. coli* findings for 43 plants for which plate- and direct-count distribution ranges were published, McFarlane *et al.* (1947), are given (Table 1). Percentages of contaminated samples for individual plants in this group ranged from 11 to 84 per cent. *E. coli* was isolated from 50 per cent or more of the samples examined from each of 25 listed plants.

TABLE 2

Incidence of E. coli in Egg-Powder Samples, by Months

Month	Number plants contributing samples	Number samples examined	Samples positive in one or more dilutions		Number of samples from which *E. coli* was isolated in dilution of			
					1:10	1:100	1:1000	1:10,000
1943				*pct.*				
Sept......................	14	118	32	27.1	13	12	5	1
Oct........................	29	217	147	67.7	58	56	29	4
Nov........................	29	323	188	58.2	98	52	31	7
Dec........................	29	215	104	48.4	58	32	11	3
1944								
Jan........................	58	321	149	46.4	76	51	20	2
Feb........................	1	2	....	....	....	....	....	....
March.....................	53	212	38	17.9	29	8	....	1
April......................	65	531	122	23.0	76	37	6	3
May........................	68	566	176	31.1	109	52	12	3
June.......................	68	567	235	41.4	116	90	24	5
July.......................	63	523	280	53.5	130	102	43	5
Aug........................	65	604	403	66.7	175	167	54	8
Sept......................	64	475	355	74.7	133	150	60	12
Oct........................	59	380	290	76.3	98	125	57	10
Nov........................	41	222	158	71.2	56	59	41	2
Dec........................	27	134	106	79.1	41	35	25	5
Total	94	5,410	2,783	51.4	1,266	1,028	418	71

Incidence of E. coli in Samples of Egg Powder, by Months: Monthly variations in the incidence of *E. coli* contamination are shown (Table 2). In 1944 it increased progressively from 18 per cent in March to 79 per cent in December, with the exception of a slight drop in November. Comparative data were not obtained for February. The degree (density) of *E. coli* contamination as indicated by the more frequent isolation of *E. coli* from the higher (1:1000 and 1:10,000) dilutions was greater in the late summer and fall months. There was little parallelism in trends in 1943 and 1944 during the months of September, October, November, and December. High shell-egg production in 1944 and overtaxed cold storage and freezing storage facilities may partially account for the differences noted in the two years.

There is good reason to believe that changing climatic conditions may have been only secondarily responsible for the seasonal trends. A comparison of the data (Table 2) with Bureau of Agricultural Economics monthly figures (1943-1944) for the sources of liquid egg used for drying reveals a close correlation between the incidence and degree of *E. coli* contamination and the kind of liquid egg dried. That is, the incidence and degree of *E. coli* contamination in the samples increased as the percentage of the storage shell- and frozen-egg liquid dried increased.

TABLE 4

Incidence of E. coli in Egg-Powder Samples According to Age

Age	Number samples examined	Samples positive in one or more dilutions		Number of samples from which *E. coli* was isolated in dilution of			
				1 : 10	1 : 100	1 : 1000	1 : 10,000
days			*pct.*				
2	8	4	......	1	1	1	1
3	62	38	61.3	12	11	11	4
4	207	121	58.5	48	43	27	3
5	492	280	56.9	101	111	57	11
6	842	457	54.3	195	194	66	2
7	936	500	53.4	239	182	66	13
8	689	353	51.2	175	126	44	8
9	537	276	51.4	127	96	46	7
10	398	188	47.2	98	64	20	6
11	307	129	42.0	54	50	21	4
12	221	98	44.3	54	30	12	2
13	137	59	43.1	31	19	8	1
14	65	27	41.5	14	13	0	0
15	30	13	43.3	7	6	0	0
16	42	17	40.5	7	8	1	1
17	33	9	27.3	5	4	0	0

Incidence of E. coli in Samples of Egg Powder, by U. S. Divisions: Most of the egg-dehydration plants were located in the midwestern area of the United States, and most of the samples examined came from there. For this reason, *E. coli* findings were summarized (Table 3) only for the three midwestern divisions—East North Central, West North Central, and South Central. Egg-powder samples from the South Central Division showed a slightly higher incidence and a slightly higher degree of *E. coli* contamination. The incidence, in per cent, for each of the three divisions was South Central, 59; East North Central, 50; and West North Central, 45. The mean ages in days of samples received from each of the divisions in the order just given were 8.9, 8.2, and 7.5, respectively.

Incidence of E. coli According to Age of Egg-Powder Samples: Egg-powder samples whose ages could be determined were grouped according to their age in days. Their mean age was 8.3 days. *E. coli* findings for the two- to 17-day groups are summarized (Table 4). In general, the older the age group the lower the incidence and degree of *E. coli* contamination in the samples within the group. The percentage of samples contaminated ranged from 61 for the three-day to 27 for the 17-day group. There were 110 samples ranging in age from 18 to 43 days. *E. coli* was isolated from 35 per cent of them.

TABLE 3

Incidence of E. coli in Egg-Powder Samples Received From East North Central, West North Central, and South Central Divisions of United States

Divisions	Number plants contributing samples	Number samples examined	Samples positive in one or more dilutions		Number of samples from which E. coli was isolated in dilution of			
				pct.	1:10	1:100	1:1000	1:10,000
East North Central (Ohio, Ind., Ill., Mich., and Wis.)	23	1,416	711	50.2	342	251	103	15
West North Central (Minn., Iowa, Mo., N. Dak., S. Dak., Nebr., and Kans.)	36	1,435	642	44.7	305	245	83	9
South Central (Ky., Tenn., Ala., Miss., Ark., La., Okla., and Texas)	25	2,088	1,228	58.8	538	463	187	40

TABLE 5

Average Plate and Direct Counts in Egg-Powder Samples According to Degree of E. coli Contamination

Count	E. coli negative all dilutions		E. coli positive 1:10		E. coli positive 1:100		E. coli positive 1:1000		E. coli positive 1:10,000	
	Number of samples	Average count	Number of samples	Average count	Number of samples	Average count	Number of samples	Average count	Number of samples	Average count
Plate	2,627	275,000	1,266	268,000	1,028	348,000	418	407,000	71	501,000
Direct	2,439	17,600,000	1,225	14,000,000	1,005	13,000,000	408	12,700,000	70	7,500,000

Relationship Between Bacterial Counts and E. coli Content: Plate and direct microscopic counts were grouped according to the degree of *E. coli* contamination in the samples; arithmetical averages for the groups are given (Table 5). Plate-count averages varied directly and direct-count averages inversely with the degree of *E. coli* contamination.[2] These relationships remained in evidence when the counts were similarly grouped and averaged for each of the midwestern divisions. They were considerably less regular when the calculations were limited to the findings for a single dehydration plant. In fact, in individual plant studies where relatively few samples were involved, it was seldom possible to detect an orderly relationship between the magnitude of the direct-count averages and the degree of *E. coli* contamination.

TABLE 6

*Incidence of Salmonella in Egg-Powder Samples According to Degree of
E. coli Contamination*

Samples	E. coli negative all dilutions	E. coli positive 1:10	E. coli positive 1:100	E. coli positive 1:1000	E. coli positive 1:10,000	Total
Number examined................	2,185	1,015	838	331	61	4,430
Number of *Salmonella* +....	589	359	374	165	28	1,515
Per cent *Salmonella* +........	27.0	35.4	44.6	49.8	45.9	34.2

Treatment of the data in terms of the WFA unofficial tentative plate-count and direct-count standards encouraged in 1943-1944, and in terms of those incorporated in the 1945 purchase specifications, reveals the comparative incidence of *E. coli* contamination in samples which met each of the standards and in those which did not. There were 4,313 samples which had plate counts less than 300,000 organisms per gram. Forty-nine per cent of them were *E. coli* positive. Of the 1,097 samples whose plate counts were greater than the standard, 60 per cent were *E. coli* positive. Direct counts were available for only 5,147 of the samples. There were 3,775 samples with direct counts less than 10,000,000 organisms per gram and 1,372 with direct counts greater than that number. In both groups of samples 53 per cent were *E. coli* positive. This percentage relationship showed little change when the higher (1945) direct-count standard was used as a basis for grouping. There were 4,235 samples with direct counts less than 15,000,000 organisms per gram and 912 with direct counts greater. Fifty-three per cent of the samples whose direct counts were less than the standard were *E. coli* positive. Forty-nine per cent of the samples whose direct counts were greater than the standard were *E. coli* positive.

Relationship Between E. coli and Salmonella Contamination: Data were available, Solowey *et al.* (1947), on the incidence of *Salmonella* in

[2] Omission from the calculations of Plant No. 20's unusually high counts, McFarlane *et al.* (1947), would not have materially changed the over-all picture (Table 5), except to have made the relationships even more apparent. The plate-count averages would have become 221,000, 256,000, 285,000, 356,000, and 501,000 and the direct-count averages 16,200,000, 13,600,000, 11,600,000, 11,100,000, and 7,500,000 organisms per gram, respectively.

4,430 of the egg-powder samples examined for *E. coli*. *Salmonella* was isolated from 589 *E. coli*-negative and from 926 *E. coli*-positive samples. Data show that the higher the degree of *E. coli* contamination in the samples, through the 1:1000 dilution, the higher the percentage of samples that contained *Salmonella* (Table 6). Gibbons and Moore (1944) also isolated *Salmonella* from *E. coli*-negative powders. They did not detect any relation between the degree of coliform or *E. coli* contamination and the presence of *Salmonella* organisms.

The frequency distribution of 14 of the more commonly isolated *Salmonella* types in relation to the degree of *E. coli* contamination in the egg-powder samples is shown (Table 7). More than half of the isolations of *S. pullorum*, *S. senftenberg*, *S. newington*, and *S. london* were made from *E. coli*-negative samples.

TABLE 7

Isolations of Salmonella Types Arranged According to Degree of E. coli Contamination in Egg-Powder Samples

Salmonella type	*E. coli* negative all dilutions	*E. coli* positive 1:10	*E. coli* positive 1:100	*E. coli* positive 1:1000	*E. coli* positive 1:10,000	Total number isolations
Pullorum	200	78	81	25	4	388
Oranienburg	94	74	99	46	8	321
Montevideo	74	31	39	23	5	172
Tennessee	43	32	34	18	2	129
Anatum	42	29	28	18	2	119
Bareilly	23	18	11	9	1	62
Typhimurium	18	10	10	12	2	52
Meleagridis	15	10	10	3	....	38
Newport	4	12	9	3	1	29
Senftenberg	18	5	2	1	1	27
Give	9	9	8	....	....	26
Newington	16	5	3	1	1	26
London	11	5	4	....	....	20
Derby	8	7	4	....	1	20
All others	34	40	46	17	3	140
Total	609	365	388	176	31	1,569[1]

[1] Isolations of two *Salmonella* types were made from some samples.

DISCUSSION OF RESULTS

Factors affecting the general microbial quality of the spray-dried whole-egg powder examined have been discussed in preceding papers of this series.

The observation that the magnitude of the plate-count averages varied directly with the degree of *E. coli* contamination of the samples (Table 5) was not entirely unexpected. Correlation between the plate counts and the presence of coliform organisms has been noted by Bartram and Black (1937) in raw milk, by Martin, Nelson, and Caulfield (1940) in commercial ice creams, and by Schneiter, Bartram, and Lepper (1943) in frozen eggs. Johns (1944), on the other hand, reported, "The determination of coliform organisms or of *E. coli* in egg powder has not been found particularly helpful." He did not note any close parallel between total counts and the presence of coliform organisms or of *E. coli*. It is true that in so far as

the individual sample is concerned a high plate count may not mean a high degree of *E. coli* contamination or even that E. coli cells are present. Data for a large number of *E. coli*-positive samples in this investigation, however, demonstrated a close parallel between the magnitude of the plate-count average and the degree of *E. coli* contamination. This is understandable, since both tests determine estimates of numbers of viable cells. The correlation between the plate counts and the *E. coli* findings would probably have been greater had the contamination always been entirely attributable to the initial quality of the raw material.

Neither a low nor a high direct count was any assurance that a given sample of egg powder was contaminated with E. coli. The inverse relationship noted in the over-all study between the magnitude of the direct-count averages and the degree of *E. coli* contamination of the samples may be explained by faulty processing technics, which favored the development of other bacterial species. Under such circumstances the *E. coli* cells initially present might die or at least become increasingly difficult to demonstrate as cells of other microbial species increased. A preponderance of paired cocci was seen on inspection of the stained films prepared from high-count samples. Johns and Berard (1945) have stated, "High microscopic counts due to the eggs themselves are largely, if not entirely, made up of rod forms; those due to faulty plant practices, on the other hand, are usually made up mainly of paired cocci, resembling the picture obtained in souring milk."

A comparison of findings for the three midwestern divisions disclosed that samples of egg powder from the South Central Division had the lowest plate-count average, McFarlane *et al.* (1947); the highest incidence of *Salmonella* contamination, Solowey *et al.* (1947); and the highest incidence and degree of *E. coli* contamination. It is possible a higher percentage of shell eggs produced in the South Central Division were soiled with feces because of outdoor feeding and nesting. In addition, temperature and humidity in the South may have been more favorable for the survival of enteric organisms. It is also possible that *Salmonella* and *E. coli* were more readily isolated from the South Central Division samples because of their lower bacterial counts.

The fact that *Salmonella* was isolated from *E. coli*-negative as well as from *E. coli*-positive egg-powder samples once again emphasizes the limitations of the *E. coli* test in determining the presence or absence of other enteric organisms.

SUMMARY

E. coli was isolated from 51 per cent of 5,410 samples of high-moisture (four to six per cent), spray-dried whole-egg powder. Approximately 91 per cent of the samples met the War Food Administration unofficial tentative standard, that is, *E. coli* was absent in 0.001 gram of egg powder. Eleven to 84 per cent of the samples examined from 43 dehydration plants were *E. coli* positive. With the exception of a slight drop in November, there was a progressive increase—18 to 79 per cent—in the monthly incidence of contamination from March through December, 1944. Increases in the monthly incidence closely paralleled increases in the percentage of

the storage shell- and frozen-egg liquid dried. Fifty-nine per cent of the samples examined from the South Central Division of the United States, 50 per cent from the East North Central Division, and 45 per cent from the West North Central Division were *E. coli* positive. The incidence of contamination was lower in samples in the older age groups, ranging from 61 per cent for the three-day to 27 per cent for the 17-day group. The degree (density) of *E. coli* contamination varied in the same manner as the incidence.

When bacterial counts were grouped according to the degree of *E. coli* contamination in the samples, their plate-count averages varied directly and their direct-count averages inversely with the degree of *E. coli* contamination.

The *E. coli* test was of little value in determining the presence or absence of *Salmonella*. *Salmonella* organisms were isolated from 589 *E. coli*-negative and from 926 *E. coli*-positive samples.

ACKNOWLEDGMENT

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COOLING OF BLANCHED VEGETABLES AND FRUITS
FOR FREEZING

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The adequacy of methods used in the commercial freezing industry for cooling fruits and vegetables following blanching (or scalding) and even the blanching treatment itself has been questioned by Magoon and Culpepper (1924), Diehl, Campbell, and Berry (1936), Williams (1945), Cruess (1946), and Woodroof, Atkinson, Cecil, and Shelor (1946). Many other investigators have studied the results of blanching practices in connection with canning and dehydration. While it is well recognized that prompt and complete cooling is necessary for the retention of vitamins, other nutritive elements, and/or flavor, Tressler (1938), the effect upon desirable qualities of the product of the method of accomplishing this aim is not known. Investigations in this field usually have not differentiated between losses obtained in blanching and those in subsequent cooling. Severe leaching losses are physiologically possible only after death of the cell and are expected to be greater in later stages of blanching and during cooling. Cooling after blanching may be done with running water (refrigerated or not), water sprays, fog or mist sprays, by air blast, or combinations of these methods. In most commercial operations fluming with cold water is used to cool as well as to convey the product to the inspection belts. In fluming, leaching of considerable quantities of soluble nutrients occurs with certain products, particularly cut or diced fruits. Air cooling has been suggested by Joslyn and Cruess (1929), Joslyn (1942), and Cruess (1946) as a more desirable procedure. Engineering difficulties and slower cooling with air have retarded extensive use of air-blast cooling.

The data presented in this paper were collected in the laboratory and in several frozen food-processing plants for the purpose of obtaining information on the actual losses occurring under different conditions of blanching and cooling. Some data on cooling rates under several conditions is presented.

In making these studies, considerable thought was given to a suitable reference base for use in expressing the experimental results. Several different approaches were used. The results, therefore, are expressed in several ways, as indicated in the text. If the changes are expressed on the wet basis, errors may be introduced: (1) by dilution with variable amounts of moisture adhering to the samples, (2) by changes in composition owing to differential solution of various constituents, and (3) by weight changes

occurring in the processed material, Adam and Stanworth (1941) and Wager (1946). If losses in soluble solids are expressed as per cents of the total dry matter, error arises through the fact that the dry matter is itself not constant, since the soluble material whose loss is being studied makes up part of the total dry matter. Therefore, changes expressed as percentages of dry matter may cause the losses to appear smaller than the actual. At the outset it seemed reasonable to assume that the water-insoluble solids of the plant tissues would be constant at all stages of processing, and that, therefore, a decrease in the ratio $\dfrac{\text{soluble solids}}{\text{insoluble solids}}$ could be regarded as an adequate measure of loss of soluble solids during processing. This was found to be a useful basis for vegetables, such as spinach and asparagus, in which the insoluble solids represent approximately half of the total solids; but for fruits, which have a much smaller proportion of insoluble solids, this ratio may not reliably represent the processing losses. In such products the insoluble solids appeared to be lost at the same rate as the soluble solids. This effect may have been caused by changes in moisture content at the various processing stages or by actual changes in the physical state of the plant tissues. While these studies were in progress Lee's (1945) report suggesting use of alcohol-insoluble solids as a reference base appeared and some of our more recent results have been expressed on this basis.

EXPERIMENTAL PROCEDURE

The samples used for analysis were either prepared in the laboratory, using blanching and cooling procedures which simulated the commercial, or through the generous co-operation of several commercial packers, were taken directly from the processing lines at appropriate points. Each figure given in the tables represents the average of from two to six samples. The samples were frozen in tightly sealed containers and kept frozen until analyses could be made. They were then either allowed to thaw and come to room temperature in tightly sealed containers to avoid changes in moisture content during thawing or they were ground at —17.8°C. (0°F.) in a Ruswin fine-bladed food chopper. A few series of vegetable samples were ground in a Waring blendor by adding the frozen material to a suitable volume of water. (It was not necessary to know the proportion of water since it would have little effect upon the ratio of $\dfrac{\text{soluble solids}}{\text{insoluble solids}}$).

Total solids were determined by drying to constant weight at 70°C. (158°F.) in a vacuum oven. Soluble solids were estimated from the refractometer readings on the liquids prepared by filtering or squeezing the macerated sample through several layers of cheesecloth. These were expressed as per cent of sucrose on the assumption that the chief component was sugar and that the refractive index was not altered by changes in composition during treatment. The insoluble solids were calculated as the differences between total and soluble solids. Alcohol-insoluble solids were determined by weighing 20 grams of the well-macerated sample into a 600-ml. beaker covered with a watch glass, simmering for 30 minutes in 80 per cent alcohol, and otherwise proceeding as described in the A.O.A.C.

(1945) method for water-insoluble solids (Section 26.7). Ash was determined by the official A.O.A.C. (1945) method (Section 34.9). Reduced ascorbic acid was determined on some of the samples by titration with suitable aliquots of a 40-gram sample blended in fresh one per cent metaphosphoric acid, with 2,6 dichlorophenolindophenol.

EXPERIMENTAL RESULTS AND DISCUSSION

A typical laboratory experiment in which a leafy vegetable, spinach, was blanched and cooled under various conditions is presented (Table 1).

TABLE 1

Comparison of Effects of Several Blanching and Cooling Procedures on Losses of Soluble Solids From Spinach [1]

Blanching treatment	Cooling treatment	Ratio $\dfrac{\text{solid solids}}{\text{insoluble solids}}$	Percentage loss of soluble solids relative to insoluble solids
None (control)	None	1.05	......
Steam blanch (90 sec.)	Air cooled	0.95	9.5
Steam blanch (90 sec.)	H$_2$O cooled (2 min.)	0.83	20.9
Steam blanch (90 sec.)	H$_2$O cooled[2](3 min.)	0.82	21.9
Steam blanch (90 sec.)	H$_2$O cooled[2](4 min.)	0.78	25.7
Water blanch (212°F., 60 sec.)	Air cooled	0.75	28.5
Water blanch (212°F., 60 sec.)	Water cooled[2](2-4 min.)	0.69	31.4

[1] Data represent averages of two or more laboratory experiments. [2] Running water.

For each experiment, 500 grams of fresh spinach was blanched and cooled as described in the table. The losses are expressed as percentage loss of soluble solids, with insoluble solids as the reference base. Steam-blanched, air-cooled spinach lost approximately 10 per cent, while water cooling increased the loss to 20 or 25 per cent, depending upon the time the material was allowed to remain in the water. When water blanching was used, the method of cooling had little effect upon the total losses. It is also interesting to note that the losses from steam-blanched spinach cooled in running water were lower than those from water-blanched spinach.

The problem of accurately expressing processing losses may be approached in another way, exemplified in the data (Table 2). In this laboratory experiment the unavoidable and variable changes in moisture content which result from the several processing steps were taken into account by starting each experiment with a known weight (300 grams) of clean, air-dry, fresh spinach. This was then processed by the particular procedure described and weighed again. After freezing and grinding (at 0°F.) and thoroughly mixing the entire sample, aliquots were taken for analysis. The calculations were made to express the total weight of the particular constituent in the entire sample. The percentage losses could then be calculated directly as per cent of the quantity present in the original sample; for example, 300 grams of fresh spinach contained 32.2 grams of total solids. After steam blanching for 90 seconds and water cooling for two minutes a similar sample of spinach weighed 314 grams

and contained 28.97 grams of total solids. This indicated a loss of 10 per cent of the total solids present in the original 300-gram lot of spinach. This procedure was satisfactory for laboratory experiments, but in commercial processing plants it was not possible to send a small weighed sample through the lines and recover it at the end. Therefore, the last column was added (Table 2), in which the losses are expressed as per cent loss in the ratio $\frac{\text{soluble solids}}{\text{insoluble solids}}$. The results expressed in this manner closely parallel the actual losses of soluble solids.

TABLE 2

Leaching Losses Occurring During Processing of Spinach for Freezing[1] Compared With Loss in the Ratio of Soluble Solids to Insoluble Solids[2]

Treatment of sample	Ash	Total solids	Soluble solids	Ratio $\frac{\text{soluble solids}}{\text{insoluble solids}}$
	pct.	*pct.*	*pct.*	*pct.*
Control (no treatment)	0	0	0	0
Steam blanched 90 sec., air cooled	8.1	5.8	11.0	10.0
Steam blanched 90 sec., water cooled 2 min.	19.3	10.01	16.9	16.3
Steam blanched 90 sec., water cooled 4 min.	23.3	10.01	20.1	20.9
Water blanched 1 min., 212°F., air cooled	25.6	11.8	30.3	35.4
Water blanched 1 min., 212°F., water cooled 2 min.	27.3	12.7	37.7	45.4

[1] Expressed as per cent of the total absolute amount of the respective constituent in a known weight of sample.   [2] Figures represent averages of duplicate experiments.

Results of a series of analyses made on asparagus taken from a commercial processing line at appropriate points are summarized (Table 3).

TABLE 3

Effect of Steam Blanching, Spray Cooling, and Fluming Upon Ratio of Soluble Solids to Insoluble Solids in Asparagus Samples Taken From a Commercial Line[1]

Series letter	Controls, ratio $\frac{\text{sol. solids}}{\text{insol. solids}}$	Blanched samples		Blanched and spray cooled		Blanched, spray cooled, and flumed	
		Ratio	Loss of sol. solids	Ratio	Loss of sol. solids	Ratio	Loss of sol. solids
			pct.		*pct.*		*pct.*
a	1.86	1.75	6.0	1.70	8.6	1.04	44.1
b	1.85	1.53	17.3	1.32	28.7	1.03	44.3
c[2]	1.84	1.62	12.0	1.58	14.1	1.63	11.4
d	1.54	1.33	13.6	1.18	23.3	1.18	23.3
e[3]	1.12	0.76	49.7	0.62	58.7	0.72	52.3
f[4]	2.04	1.48	27.2	......	......	1.35	34.2
g[4]	1.25	1.11	47.7	......	......	1.04	50.9

[1] Figures represent averages of duplicate samplings.   [2] Center cuts of asparagus.   [3] Samples cooked before analysis (approximately one and one-half pounds frozen asparagus cooked in one quart of water for 12 minutes; cooking water discarded).   [4] Samples f and g were laboratory processed samples, blanched three and one-half minutes in steam and cooled three minutes in running water.

The procedure followed by this plant was cutting, washing, sorting, grading, steam blanching, spray cooling, fluming, packaging, freezing. The (a) series was taken early on a Monday morning before the steam pressure had reached the desired point. This could explain the low blanching losses of this series compared with the others. However, after fluming the soluble

solids had dropped as low as those of the "b" series, which represented the average or "typical" condition over this day's run. A gradual loss at each successive stage of processing was observed in this asparagus. Series "c," which was "center cuts," lost soluble solids during blanching but no further loss occurred during cooling. The tender tips underwent greater losses at the various stages than the stalks. The series "d" and "e," are representative of samples taken from the line on the following day. The asparagus was of the same lot which had merely been held overnight. The lower ratio for the controls on the "d" series shows that the asparagus had undergone losses in soluble solids during storage. These changes appear to have made the asparagus more resistant to leaching during fluming treatment. The "e" series was similar to "d" except that each sample was cooked (approximately 1½ lb. frozen asparagus cooked in one qt. water for 12 min.) before analysis. The cooking water was discarded, as is frequently done. The cooking losses were over twice as great as the processing losses, and there was little difference among the samples at the various stages of processing, indicating that cooking losses, in vegetables (if cooking water is discarded) could have a leveling effect on differences in processing losses. The laboratory samples of the "g" series showed a similar effect of cooking.

Series "f" and "g" represent laboratory samples which were processed the same morning that they were cut. These samples had a higher initial soluble-solids content than the commercial. They lost a high percentage of soluble material during steam blanching, probably because they were more tender than the commercial, and because the blanching treatment used was perhaps unnecessarily severe. (Peroxidase was inactivated, while it was still present in the commercial samples.)

The young inflorescence, which is the edible portion of the broccoli plant, represents still a third type of vegetable which was included in this study; data of an experiment conducted in a commercial plant are shown (Table 4). Samples of broccoli were taken from the line at the points indicated in the table and cooled in air or in large kettles of cold water under laboratory conditions. The water was changed for each sample to simulate the effect of running water. For the water-blanched samples, cleaned broccoli was removed from the processing line and both blanching and cooling were done in the laboratory. The data (Table 4) show that steaming followed by air cooling caused the least loss of soluble solids. As soon as water was introduced either for scalding or for cooling of broccoli, the losses of soluble solids increased. The data for vitamin C presented a different picture. Whether expressed on the wet basis or the alcohol insoluble-solids basis or the soluble-solids basis, the loss of this vitamin was nearly as great when air cooling was employed. This is probably explained by the destruction of the vitamin brought about by the longer exposure to heat in the case of the air-cooled samples compared with the water-cooled, as has been shown by Tressler (1938).

Diced carrots were studied as an example of a root vegetable. They also represent one which has a higher ratio of soluble solids to insoluble solids than other vegetables used in our studies. The average of four sam-

TABLE 4

Effect of Scalding and Cooling Procedure on Soluble Solids and
Vitamin C Content of Frozen Broccoli

Treatment of sample	Ratio sol. solids / insol. solids	Loss soluble solids	Vitamin C					
			Wet basis	Loss (wet basis)	Alc. insol. solids	Loss (alc. insol. solids basis)	Insoluble solids basis	Loss (insoluble solids basis)
		pct.	mg./gm.	pct.	mg./gm.	pct.	mg./gm.	pct.
Washed and drained............	2.0	.....	1.51	....	25.1	....	38	....
Steamed 3 min., air cooled............	1.9	5	1.28	15.2	18.8	25	32	15.7
Steamed 3 min., water cooled 1 min............	1.4	30	1.16	23	18.7	25	29	23
Steamed 3 min., water cooled 5 min............	1.4	30	1.13	25	19.1	24	28	26
Boiling water 3 min., air cooled............	1.5	25	1.13	25	18.8	25	28	26
Boiling water 3 min., water cooled 1 min............	1.4	30	1.13	25	19.1	24	28	26
Boiling water 3 min., water cooled 5 min............	1.3	35	1.01	33	16.8	33	25	34
Commercial process (steamed 3 min., cooled in running water)............	1.2	40	1.13	25	18.5	26	28	26

ples taken at each point from a commercial processing line (Table 5) shows that the content of alcohol-insoluble solids decreased on scalding more than expected. This effect may have been caused by absorption of moisture during steaming. The apparent increase in alcohol-insolubles after spray cooling may be explained on the basis of loss of soluble solids. Assuming that alcohol-insoluble solids are the best reference base, the ratio of soluble solids to alcohol-insoluble solids should be the best reflection of the effect of processing upon the soluble nutrients. The ratios show that no loss occurred during steam blanching, but water cooling brought about a significant loss. The ratios of soluble solids to insoluble solids (obtained by difference between total and soluble solids) present a different picture. The sharpest drop here occurred as a result of blanching rather than of water cooling.

TABLE 5

Effect of Processing on Soluble-Solids and Alcohol-Insoluble Solids Content of Diced Carrots[1]

Processing stage	Total solids	Soluble solids (wet basis)	Alcohol-insoluble solids (wet basis)	Ratio soluble solids / insoluble solids	Loss	Ratio soluble solids / alcohol-insol. solids
	pct.	pct.	pct.		pct.	
Raw, diced................	13.69	10.93	5.90	3.96	....	1.85
Blanched (steam)....	13.47	10.04	5.02	2.93	26	2.00
Spray cooled.............	11.95	8.66	6.19	2.63	33	1.40
Frozen......................	13.43	9.97	6.74	2.85	27	1.48

[1] Commercial samples; averages of four samples.

TABLE 6

Effect of Processing on Water-Soluble Constituents of Lima Beans for Freezing[1]

Constituent	Fresh untreated		Steam blanched 3½ min.		Steam blanched and flumed	
	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis
Total solids per cent).............................	34.0	....	32.7	....	29.7	....
Total sugars (pct. dextrose)....................	2.18	6.5	1.86	4.7	1.44	4.8
Riboflavin (μg./gm.)..............................	0.70	1.9	0.58	1.7	0.53	1.7
Thiamin (μg./gm.)................................	1.21	3.4	0.81	3.1	0.94	2.5

[1] Commercial samples; average analysis of four samples.

Lima beans are an example of a vegetable having a thick protective coat which should reduce the leaching losses. The relatively high proportion of starch and low proportion of soluble carbohydrates also distinguishes it from the other vegetables discussed. The presence of this starch may also play a role in keeping the blanching losses low since the starch just below the seed coat, which was exposed to heat, would swell and form a less permeable layer. The ratio of soluble solids to insoluble solids in lima beans is very low and did not prove to be of value in showing processing losses. The data (Table 6) summarize the results of the analyses of a series of samples taken from a commercial lima bean-processing line. It will be noted that the total solids decreased as a result of blanching and cooling.

This decrease probably was not real but was caused by moisture uptake by the starch in the beans. The data for ash, total sugars [determined by Hassid's (1936) ceric sulfate method], thiamin [determined by Conner and Straub's (1941) method], and riboflavin [by Mackinney and Sugihara's (1942) modification of the fluorometric procedure] have been expressed on both the fresh- and dry-weight basis. Owing to the relatively high proportion of starch and low proportion of soluble solids, the dry-matter basis is probably the more reliable in this instance. Unfortunately alcohol-insoluble solids were omitted from these determinations.

Frozen fruits for use in bakery products or preserves may be blanched before freezing, for color and flavor retention. If the fruit is blanched, the losses in soluble solids which may occur are even greater than in vegetables largely because of the relatively higher sugar content, lower insoluble-solids content, and the soft nature of the tissues. Furthermore, in products prepared from such blanched fruit, losses of the highly volatile and soluble flavor constituents are much more noticeable than in vegetables.

TABLE 7

Effect of Processing on Soluble Solids Content of Sliced Apples [1]

Processing stage	Total solids	Soluble solids (wet basis)	Ratio sol. solids insol. solids	Loss of soluble solids	Alcohol-insoluble solids (wet basis)	Ratio soluble solids alc.-insol. sol.	Soluble solids loss
	pct.	*pct.*		*pct.*	*pct.*		*pct.*
Raw (peeled and sliced)	17.42	14.89	5.88	....	2.44	6.10	....
Raw (flumed to blancher)	16.18	13.65	5.39	8.3	2.24	6.09	0.2
Blanched (6 min. at 190°F.)	13.72	11.82	5.30	9.9	2.23	5.30	13.1
Spray cooled (5 min.)	12.93	10.64	4.50	23.5	2.13	5.00	18.0
Flumed (64-68°F.)	12.35	10.12	4.53	23.0	2.06	4.92	19.4

[1] Commercial samples; four samples were taken from each point.

Data on apples obtained from a commercial line are summarized (Table 7). In these the alcohol-insoluble solids were a reasonably constant reference base for calculation of the losses occurring during processing. It will be seen that blanching itself caused the greatest loss of soluble material; spray cooling and fluming further increased these losses.

Similar experiments (Table 8) were made on commercial samples of sliced clingstone peaches. This is an example of a product from which insoluble solids appeared to be lost as rapidly as soluble solids, so that no processing losses could be demonstrated from the differences in the ratio of soluble solids to insoluble solids for this fruit.

COOLING RATES

Since the importance of rapid and complete cooling is well recognized, measurements were made of rates of cooling sliced apples under several laboratory conditions. The apples were rather small Newton Pippins which

were peeled, cored, sliced into eighths, and placed on a metal-frame, wire-mesh tray, using two and one-half pounds of apple slices per square foot of tray surface. An average-sized slice was selected for temperature measurement from the center portion of a tray. The hot junction of a copper-constantin thermocouple was inserted so that it passed through the thickest portion of the slice (running in a direction parallel to the core of the apple). Preliminary trials showed that the slices were completely cooked if steaming was continued until the thermocouple showed that the inside of the slice had reached 98.9°C.(210°F.). Qualitative enzyme tests indicated that three minutes in steam was a sufficient blanch for these apple

TABLE 8

Effect of Processing on Soluble-Solids Content of Sliced Cling Peaches [1]

Processing stage	Total solids	Soluble solids (wet basis)	Insoluble solids (by difference)	Ratio sol. solids / insol. solids	Soluble solids (in total solids)
	pct.	pct.	pct.		pct.
Unpeeled halves..............................	13.16	11.72	1.44	8.14	89
Lye-dipped halves.............................	13.11	11.67	1.44	8.10	89
Halves after lye and skin were washed off.............................	13.44	11.93	1.51	7.90	88
Raw slices after fluming.....................	13.29	11.86	1.43	8.29	89
Blanched, spray-cooled slices..........................	11.94	10.70	1.24	8.62	89
Blanched, spray-cooled, flumed slices.............	10.56	9.45	1.11	8.51	89

[1] Commercial sample; six samples were taken from each point.

slices. The slices usually attained a temperature of approximately 90.6°C. (195°F.) within this time. The time of steaming was counted from the time the surrounding atmosphere attained a temperature of 210°F., as indicated by a separate thermocouple placed loosely above the tray. After steaming the entire tray was removed from the steaming chamber and was cooled either in still air, by means of a four-bladed Emerson electric fan 12 inches in diameter placed at a distance of two feet from the tray so that the air current blew from above or below the tray, by immersion in a large volume of water at 12.8°C.(55°F.), or by spraying with water at that temperature. Cooling rates were measured by recording the time required (in seconds) for the temperature within the apple to drop each 10°F. as it cooled from 210 to 70°F. Such cooling rates are shown (Fig. 1); each curve was drawn from an average of five or more determinations. The data show that the cooling rate in still air is entirely too slow. However, under laboratory conditions, at least, the electric fan cooled the apples at a rate which was not markedly below the rate attained with water cooling. The data indicate that placing the fan below the tray had a slight advantage over placing it above the tray and that spray cooling had a similar advantage over immersion in a tank of water.

The above cooling rates were taken with an air temperature of 70°F. prevailing in the laboratory. It is obvious that very frequently climatic conditions during processing seasons bring about considerably higher temperatures in the surroundings; therefore, the data plotted (Fig. 2) were taken. For these cooling rates the steamed apples were placed in the
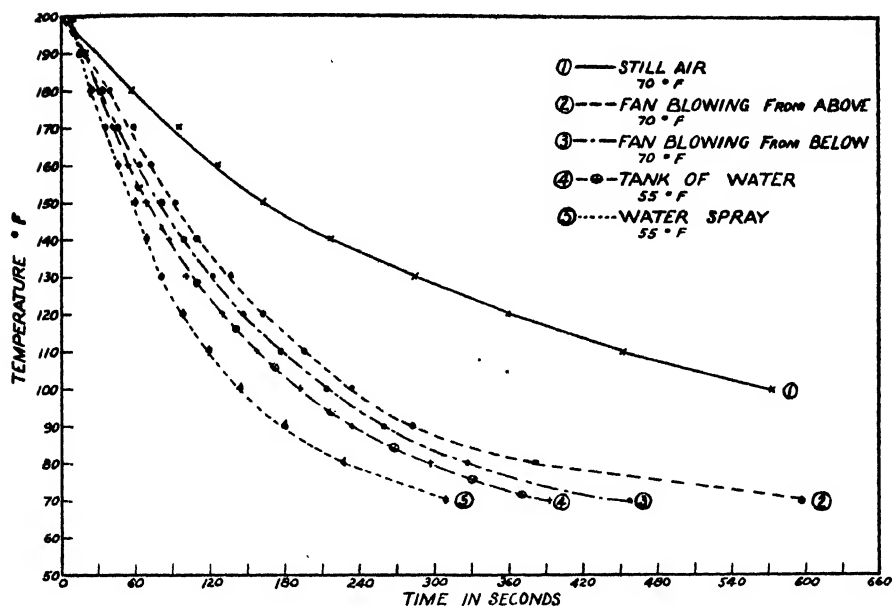
FIG. 1. Rates of cooling of sliced apples.
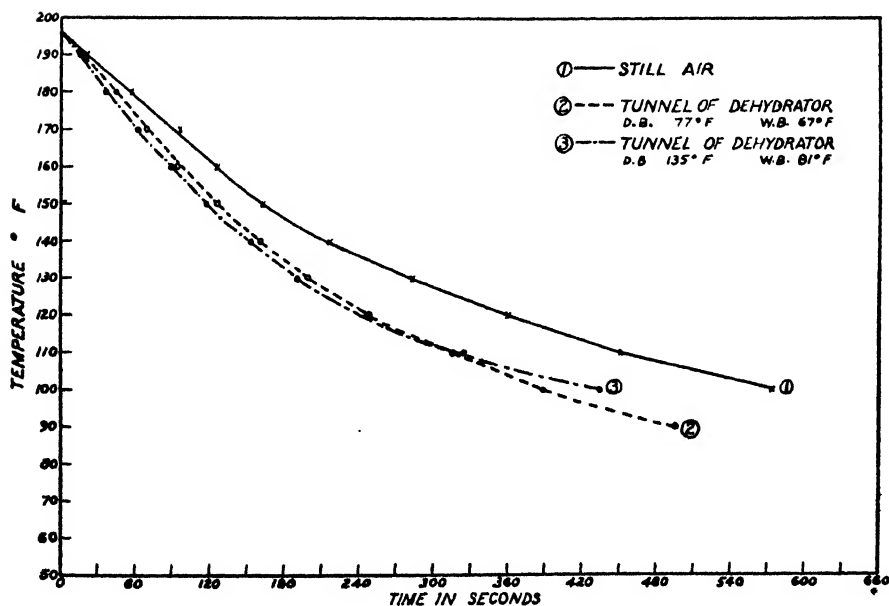
FIG. 2. Rates of cooling of sliced blanched apples in air under several conditions.

tunnel of a dehydrater with the respective wet and dry bulb temperatures as indicated on the figure. It can be observed that the cooling rates of the apple slices when placed in the moving air were very similar, regardless of temperature, until the temperature of the product approached that of the surroundings.

SUMMARY

Investigations were made of the changes in soluble nutrient content which occur in several stages of processing vegetables and fruits for freezing, under laboratory and commercial conditions. Spinach, asparagus, carrots, lima beans, broccoli, apples, and clingstone peaches were included.

Consideration was given to the most reliable reference base for expression of changes. For vegetables having relatively low soluble-solids content the insoluble solids (by difference between total solids and soluble solids) constituted a suitable basis; while in products having high soluble-solids content, the alcohol-insoluble solids were found more reliable. Probably the latter would be most suitable for general use.

Of all the blanching and cooling combinations used, steam blanching followed by air cooling brought about the least loss of soluble solids. If water blanching was used, there was little advantage to be gained by air cooling.

Prolonged contact with large volumes of rapidly running water is most detrimental to the flavor and nutrient content of such products as fruits, spinach, and carrots, which have either very soft texture or relatively very high soluble-solids content or both.

Studies of rates of cooling of sliced apples indicated that with cold, rapidly moving air the apples could be cooled almost as rapidly as with water.

ACKNOWLEDGMENTS

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VITAMIN RETENTION AND ACCEPTABILITY OF FRESH VEGETABLES COOKED BY FOUR HOUSEHOLD METHODS AND BY AN INSTITUTIONAL METHOD [1,2,3]

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Variation in the methods of cooking fresh vegetables exists among homes and institutions, and usually in any one place the same method is used for almost all vegetables. This practice undoubtedly prevails because of habit and because of *a priori* conclusions, not because a careful comparison has been made to decide which method gives the best product for each vegetable from the standpoint of acceptability as well as that of vitamin retention.

In the study reported in this paper three vegetables—green cabbage, spinach, and peas—were cooked in four-serving portions by four methods: in water to cover, in a minimum amount of water, in a steamer, and in a pressure saucepan. Then the retention of ascorbic acid, thiamin, and riboflavin and also the acceptability were determined for the cooked vegetables. In addition, the effect of storing the cooking liquid in the refrigerator and subsequently reheating it was determined. A comparison was also made of cabbage cut in quarters and shredded and of spinach with and without stems, all cooked in water to cover. Finally two of the vegetables, cabbage and spinach, were also cooked in a steam-jacketed kettle in an institution and held over steam. The vitamin retention found in these institutional products was compared with similarly cut vegetables cooked by a household method in water to cover.

A number of studies on these vegetables and their vitamin retention have been reported in the literature, but only those are reviewed in which the methods were at all comparable to those used in this investigation. Thus, for the household methods only those studies are included in which it was possible to determine that the vegetables were cooked in quantities of at least two servings and in which more than one method was reported, except in the case of cabbage cut in quarters and spinach with the stems left on.

It is difficult to draw clear-cut conclusions from the data presented in the literature for these reasons: (1) the range of results was wide between

reports on the same method and vegetable; (2) some papers showed appreciable overlapping of results; (3) some authors found differences between methods while others did not; (4) the figures on retention did not always take into account the change of weight on cooking; (5) the results on institutional methods were reported in various ways and frequently not very clearly; retentions for the combined effect of cooking and holding vegetables were given sometimes only for the solid portion, then again only for the combined solid and liquid, and often the retentions after holding were apparently based on the vitamin value of the cooked rather than the raw product; and (6) the time of holding the cooked product from the different institutions varied.

The trend in the results reported on household methods was for the drained vegetables to show the least retention when cooked in a considerable quantity of water and the greatest when cooked in a minimum quantity of water. For all four methods summarized together the retentions in the cabbage, shredded or cut in small pieces, were one-fourth to all of the ascorbic acid; in spinach, without stems in one study, one-fourth to three-fifths of the ascorbic acid, one-half to four-fifths of the thiamin, and one-half to four-fifths of the riboflavin; in peas one-half to four-fifths of the ascorbic acid, one-half to almost all of the thiamin, and two-thirds to almost all of the riboflavin. These values were taken from reports by Halliday and Noble (1936); Wellington and Tressler (1938); Lunde, Kringstad, and Olsen (1940); Brinkman, Halliday, Hinman, and Hamner (1942); Higgins (1942); MacGregor (1942); Oser, Melnick, and Oser (1943); Gleim, Tressler, and Fenton (1944); Ireson and Eheart (1944); and Noble and Waddell (1945).

For cabbage cooked in quarters in water to cover, Wellington and Tressler (1938) reported 38 per cent retention of ascorbic acid. For spinach with the stems left on and cooked in water to cover, Higgins (1942) reported 24 per cent retention of ascorbic acid.

Where values for the cooking liquid for the three vegetables were given, one-third to three-fifths of the vitamin content was dissolved in the method using a considerable amount of water, whereas only negligible amounts to one-fourth of the totals were found in the other methods of cooking.

Subjective scoring of acceptability was included with only three of the studies on retention in the household methods. In two of the reports, one by Brinkman, Halliday, Hinman, and Hamner (1942) and the other by Montgomery (1943), whose work on acceptability was done in collaboration with MacGregor's (1942) on retention, the color, flavor, and texture of all three vegetables were scored and also the odor of cabbage. These workers found that the open-saucepan method using water to cover was best for cabbage, the open-saucepan and pressure-saucepan methods best and equally good for spinach, and the latter method best for peas. The pressure-saucepan method for cabbage was scored down for color in one study and for color, flavor, and odor in another. The open-saucepan method for peas was scored high in all respects except color. The covered saucepan with a minimum amount of water and the steamer both ranked

low, although often the scores for flavor and sometimes for texture were equal to those for the first two methods. In the third report, by Gleim, Tressler, and Fenton (1944), spinach was found to have a more bland flavor when cooked in a larger amount of water than when cooked in a smaller amount; however, the 10 to 17 judges were equally divided in their choice of the two methods.

Concerning the effect of storing the cooking liquid and then reheating it, the literature contained investigations only on the ascorbic acid in cabbage liquid. MacGregor (1942) reported retentions of 86 per cent after 24 hours' storage in a refrigerator, 80 per cent after 48 hours, and 32 per cent after boiling the latter sample for 15 minutes. Lampitt, Clayson, and Barnes (1944) found 76 per cent retention after two hours at room temperature and 64 to 42 per cent in five to 20 minutes while maintaining it at 88°C.(190.4°F.).

For the results on vegetables cooked in a steam-jacketed kettle it should be stated that the method of preparation, especially cutting, was not always given exactly. Cabbage retained one-third to three-fifths of its ascorbic acid just after cooking, one-third to three-fifths after holding less than one hour, one-fourth to one-half after holding one to two hours, and 1/500 after holding more than two hours. In the same order for cooking and holding, spinach retained one-fifth to two-thirds, two-fifths to seven-eighths, one-tenth to one-third, and one-tenth of its ascorbic acid. Spinach also retained one-half to four-fifths of its thiamin and one-half to nine-tenths of its riboflavin just after cooking and one-fifth of its thiamin after holding three hours. These results are summarized from reports by Higgins (1942); Daum, Aimone, and Hollister (1943); Jenkins (1943); Nagel and Harris (1943); Cutlar, Jones, Harris, and Fenton (1944); Scoular and Bryan (1944); Smith, Caldwell, Ross, and Wood (1944); Schauss (1945); and McMillan and Todhunter (1946). No holding studies for these three vegetables were found for riboflavin, but Porter and Kelly (1944), using Swiss chard, reported no loss in one hour of holding over steam.

EXPERIMENTAL PROCEDURE

Preparation of Samples for Analyses: The green cabbage, spinach, and peas used for the household methods of cooking were purchased in a local grocery store. The cabbage heads had the outer leaves discarded, then, without washing, were cut and sampled by taking strips in rotation around the head. The spinach was washed by dipping each plant first into warm then into cold water, the stems were removed, and the leaves gently patted dry with a towel. The peas were shelled but not washed. The vegetables were tied loosely in cheesecloth bags, each containing a two-serving portion. This procedure was used in order to keep the samples intact during cooking. Because of the time involved in preparation, the bags of spinach and peas were kept overnight in a refrigerator and cooked the next day.

The vegetables were cooked in four-serving portions by these methods: (1) in an open saucepan in water to cover,[4] (2) in a tightly covered sauce-

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[4] The vegetables were cooked according to directions in *Hows and Whys of Cooking,* by Halliday and Noble (1933 Edition).

pan in a minimum amount of water,[5] (3) in a steamer on an enamel plate, therefore without added water, and (4) in a pressure saucepan in a very small quantity of water.[6] No seasoning was added. The order of cooking by the different methods was rotated so that no one method had an unfair advantage over the other. Immediately after cooking the vegetables were drained, weighed, and the total liquid cooled and measured. Some of the liquid from the open-saucepan method was allowed to stand covered in a refrigerator for one to two days and then was reheated in a tightly covered pan. Specific data for these cooking procedures are given (Tables 1 and 2).

The green cabbage and spinach used for the institutional method of cooking were purchased on the wholesale market. The cabbage heads had the outer leaves discarded, were cut in wedges, and the samples tied loosely in six cheesecloth bags, each containing a two-serving portion. The precaution was taken to see that wedges from the same heads were represented in each bag. As was the practice of the institution, the cabbage was stored in a refrigerator for about 20 hours after cutting, then washed before cooking. The spinach was cut free of any tough stems, washed first in warm then in cold water, and the portion to be analyzed was gently patted dry with a towel and tied loosely in six cheesecloth bags, each containing a two-serving portion. It was cooked the same morning as prepared.

The bagged samples, together with about a bushel of vegetable prepared by the institution, were cooked in either of two deep-type, steam-jacketed kettles of 25- or 30-gallon capacity, depending upon which utensil was available. Salt and sugar were added to the cabbage and salt to the spinach. The lid was kept closed during cooking. Immediately after cooking the bags of vegetable were removed, drained, and weighed. Two of the samples were held over steam for one and one-half hours. No attempt was made to measure the cooking liquid. Specific data for the cooking procedures are given (Table 2).

Preparation of Samples for Acceptability: For the subjective tests of acceptability (Table 1), the vegetables were washed, cut, sampled, and cooked by the household methods. Trial cookings determined the length of the cooking period so that all the samples to be scored were finished simultaneously; a minimum of three judges scored the products using score cards developed for this study. Wherever possible the color was compared with a standard color chart of Maerz and Paul (1930).

Determination of Ascorbic Acid: For determining the reduced ascorbic acid a modification which included technical details from Bessey (1938), Evelyn, Malloy, and Rosen (1938), Morell (1941), Loeffler and Ponting (1942), and Ponting (1943) was used and in brief was as follows:

All of the vegetable from one of the bags was placed in a Waring blendor with three per cent oxalic acid in the ratio of approximately four parts of acid to three parts of vegetable and blended for four to six minutes. This proportion of acid gave a pH of 1.5 to 2.1. Two aliquots from this mixture, the size of which depended on the amount of ascorbic acid in the vegetable, were then blended for one minute in enough 0.4 per

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[5] The directions of the manufacturer were followed.
[6] The FlexSeal pressure saucepan was used.

cent oxalic acid to make a total volume of approximately 250 ml. The pH of these second extracts was 1.4 to. 2.0. The total time of blending was five minutes for cabbage and peas and seven minutes for spinach. The extra precaution of using an atmosphere of nitrogen for blending, as suggested by Hochberg, Melnick, and Oser (1943), was adopted for spinach and peas although comparative experiments with all three vegetables gave no conclusive evidence that this procedure was necessary in order to protect reduced ascorbic acid when three per cent oxalic acid was used in the proportion stated. The reduced ascorbic acid was then determined on a Coleman universal spectrophotometer (Model 11).

No attempt was made to determine dehydroascorbic acid. Other workers, however, have found the three vegetables used in this study to be either low or lacking in this form of the vitamin. The most recent reports on this subject are by Harris and Olliver (1942), Gleim, Tressler, and Fenton (1944), Pavcek and Elvehjem (1944), and Roe and Oesterling (1944).

Determination of Thiamin and Riboflavin: For determining the thiamin and riboflavin a combination of the techniques of Hennessy and Cerecedo (1939), Conner and Straub (1941), Najjar (1941), and Hennessy (1942), as described for thiamin by Hinman, Higgins, and Halliday (1947) and for riboflavin by Hinman, Tucker, Jans, and Halliday (1946), was used. The essentials of this were as follows:

All of the vegetable from one of the bags was mixed with an equal quantity of water in a Waring blendor. Then aliquots of the blend were extracted with N/10 H_2SO_4, digested with clarase, adsorbed on double columns, eluted, oxidized, and the fluorescence readings for thiamin and riboflavin made on a Coleman electronic photofluorometer (Model 12).

Standard recoveries for thiamin and riboflavin, which were added to an aliquot each of a raw and a cooked vegetable blend and carried through the entire procedure, were always 90 per cent or better in each experiment.

Statistical Analyses: When three or more values were compared, the data on percentage retentions were analyzed statistically for the minimum level of significance between means at the five-per cent point as described by Noble and Waddell (1945); when two values, such as those from the stored, reheated cooking liquid of cabbage strips, by the paired "t" test; and when two values, such as those from cabbage freshly cooked in small and large quantities, by the non-paired "t" test.

Duplication of Results: In order to check the precision in sampling and in replicate cookings, one vegetable was cooked in duplicate by one method on two different days using different lots of vegetable. The vegetable chosen was spinach with the stems left on; the method was the open saucepan. It was found that in duplicate cookings from the same lot of spinach the percentage retentions of each of the three vitamins in the drained vegetable agreed within 0 to seven and that in the stored and the stored, reheated liquids the percentage retentions of thiamin and riboflavin agreed within one to nine. This range is within the experimental error in the chemical methods.

The same order of precision had been obtained for reduced ascorbic acid values when the influence of various factors, such as (1) the effects of hold-

ing the concentrated first blends made with three per cent oxalic acid plus the three raw vegetables and also of holding the filtrates of the second blends—both when mixed in the presence of air and of nitrogen and (2) the effect of the extracting solution on raw broccoli, had been tested in the analytical method. Perhaps this was because, as has been stated, such large samples were blended in the Waring blendor before aliquots were removed for the analyses.

<div align="center">DISCUSSION OF RESULTS</div>

Small-Quantity Cookings: From the results presented (Fig. 1) on the retentions of the three water-soluble vitamins in cabbage strips, in spinach
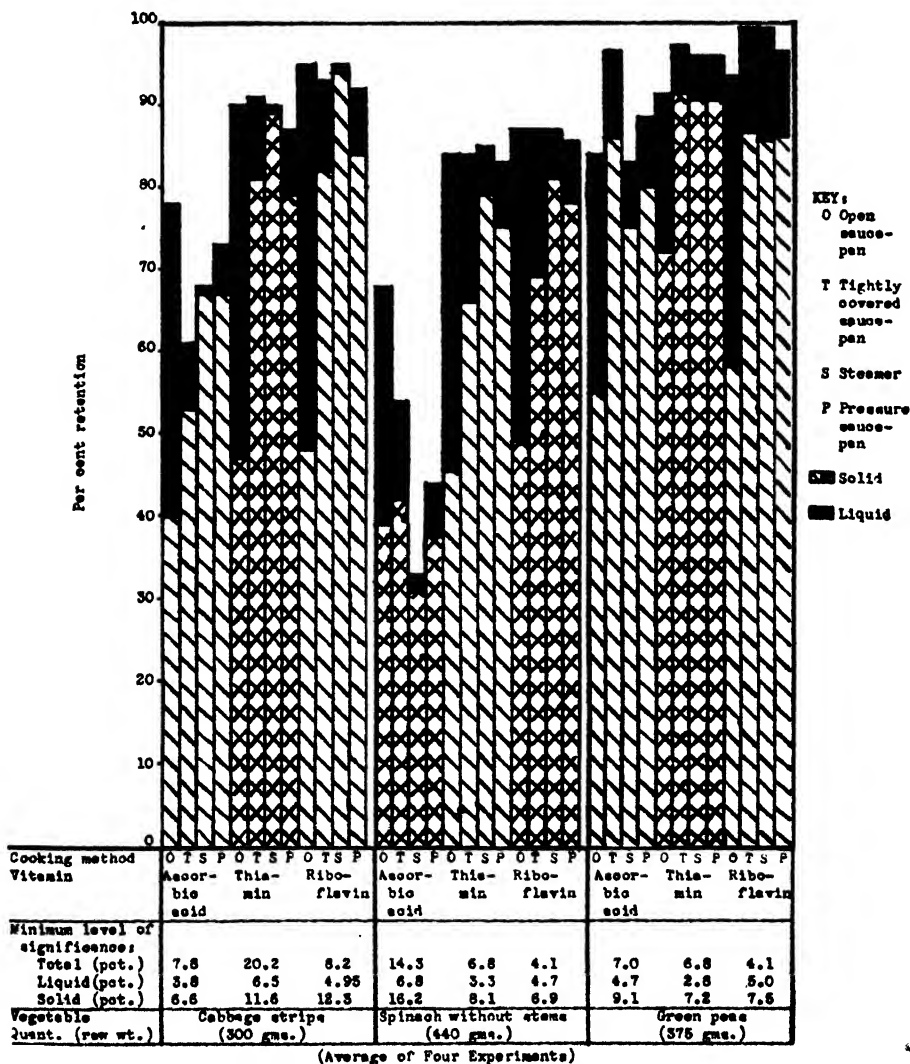
FIG. 1. Vitamin retention of vegetables cooked by four household methods.

without stems, and in peas, each cooked by the four household methods, the following conclusions can be drawn:

TABLE 3

Range of Vitamin Content of Raw Vegetables Used in Cooking Experiments [1]

Vegetable	Method of cooking	Ascorbic acid			Thiamin			Riboflavin		
		Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.
		mg./100 gm.			*μg./100 gm.*			*μg./100 gm.*		
Cabbage strips	Household	39.4	50.1	67.2	39	54	72	31	44	52
Spinach without stems	Household	16.3	40.3	59.8	130	176	226	209	259	320
Peas	Household	21.2	26.5	31.0	296	347	402	121	132	146
Cabbage wedges	Institutional	32.5	44.4	61.8	46	58	63	27	32	39
	Household	35.3	37.1	39.5	33	46	58	25	27	28
Spinach with stems	Institutional	6.6	23.6	57.1	74	99	119	137	175	206
	Household	17.8	23.2	28.6	104	110	116	193	197	201

[1] Average of four experiments.

The open-saucepan method gave the lowest retentions in the drained products, except for the ascorbic acid of spinach which was about equally well retained by all four methods. As might be expected, all the vitamins from the three vegetables were present in significantly larger proportions in the cooking liquid from the open-saucepan method than in that from any of the others. In fact, by this method the vitamins were about equally distributed between solid and liquid for two of the vegetables, cabbage and spinach, but not so for peas. In the latter the liquid had a much lower proportion than the solid.

The other three methods of cooking gave retentions for the drained, cooked vegetables which were equally good with these exceptions: (1) the tightly covered saucepan gave retentions appreciably lower in ascorbic acid for cabbage and slightly lower in thiamin and riboflavin for spinach than did the steamer and the pressure saucepan; (2) the tightly covered saucepan, on the other hand, showed a slightly higher retention for ascorbic acid in peas than did the steamer, whereas the pressure saucepan ranked in between these two and overlapped both methods. In the liquids, these three methods gave values ranging from a trace to 18 per cent of the content of the raw vegetable.

For the total retentions—drained vegetable plus liquid—all methods gave similar results for thiamin and riboflavin but showed significant differences for ascorbic acid. No one of the methods, however, consistently gave higher values than the others for all of the vegetables.

Although the literature does not contain values for a combination of all the vitamins, vegetables, and cooking methods studied here, the results in this report are in general within the range of those published. Occasionally a value for an individual experiment or the average of the four experiments was outside this range.

From the results presented (Table 4) on the subjective acceptability tests on the three vegetables cooked by the four methods, it can be said

that for cabbage the open-saucepan and the pressure-saucepan methods gave the most satisfactory results; for spinach the open-saucepan method ranked first with the steamer and the pressure-saucepan methods next; and for peas the pressure saucepan definitely gave the best results. In interpreting these conclusions one must realize that for cabbage the production of undesirable flavors may mask the retained natural flavor and that for spinach loss of some of the flavor may be considered an advantage.

TABLE 4

Judges'[1] Scores[2] for Acceptability Tests

(Highest possible score 5; lowest 1)

Vegetable	Number experiments averaged	Acceptability factors		Method of cooking			
				Open-saucepan	Tightly covered saucepan	Steamer	Pressure-saucepan
Cabbage strips	5	Color { green		4.8±.2	2.4±.2	2.4±.3	4.0±.1
		{ white		4.7±.1	3.1±.3	3.1±.2	4.5±.3
		Desirability of aroma		4.6±.3	2.7±.2	2.6±.4	3.9±.4
		Gain of flavor		5.0±.0	3.2±.5	3.0±.3	4.2±.4
		Intensity of natural flavor		2.4±.3	3.8±.3	3.6±.4	4.5±.2
		Texture		4.1±.1	3.7±.2	4.0±.1	4.1±.3
		General impression of product as a whole		3.9±.1	2.6±.3	3.2±.2	4.3±.1
Spinach without stems	3	Color		4.9±.1	2.3±.2	3.3±.5	3.6±.7
		Desirability of flavor		3.6±.1	2.7±.4	3.4±.4	3.4±.4
		Intensity of natural flavor		2.3±.2	4.0±.3	3.7±.3	4.0±.3
		Texture		4.1±.2	3.3±.4	3.5±.2	3.3±.4
		General impression of product as a whole		3.5±.4	2.5±.2	3.4±.4	3.4±.3
Peas	4	Color		3.5±.1	2.1±.45	2.2±.25	4.8±.2
		Shape		2.9±.1	2.3±.35	2.2±.25	4.0±.05
		Intensity of natural flavor		2.7±.4	3.8±.5	3.0±.1	4.1±.4
		Texture		3.8±.2	3.7±.2	3.5±.3	3.9±.3
		General impression of product as a whole		3.1±.4	2.8±.5	2.4±.25	4.1±.5

[1] Three or four judges participated in each experiment   [2] These values represent the average and the average deviation from the mean of the tests.

From the judges' opinions of each product as they considered it *in toto* the pressure saucepan and the open saucepan always ranked higher than the steamer or the tightly covered saucepan except that the steamer was equivalent to the first two methods for spinach.

When both the retentions of vitamins and the acceptability of the drained cooked vegetables are considered simultaneously, the following conclusions can be drawn:

For both green cabbage and peas, the pressure saucepan gave the best product. If a pressure saucepan is not available then the factors of retention and acceptability will have to be weighed by the individual to determine which of the three methods he would prefer, that is, the open saucepan for acceptability or the steamer and tightly covered saucepan for retention.

For spinach, the steamer and pressure saucepan gave the best products. If neither of these methods is available, then the choice would be between

the open saucepan for acceptability and the tightly covered saucepan for retention.

Stored Cooking Liquid: From the results (Table 5) on the keeping quality of the cooking liquid, it can be seen that the loss in the reduced ascorbic acid content of the liquids during storage in the refrigerator was appreciable. Cabbage liquid, stored for two days, was not tested upon removal from the refrigerator but had lost one-half of its original ascorbic acid after being brought to a boil but showed no further loss after boiling for 15 minutes. These values are within the range of those found by Lampitt, Clayson, and Barnes (1944). In the liquid from peas, storage alone caused a loss in ascorbic acid of about three-fourths in two days and in the liquid from the spinach about one-fifth in one day. Merely bringing these two liquids up to the boiling point increased these losses to four-fifths and two-fifths, respectively. No significant further change was found after boiling 15 minutes.

TABLE 5

Stability of Vitamins in Stored Cooking Liquid From Vegetables Cooked in an Open Saucepan

Source of liquid	Number of experiments	Vitamin	Time held in refrig.	Content before stored (av.)	Retention [1] (av.)			Minimum level of significance
					Just before heated	After brought to boil	After boiled 15 min.	
			days	*per 100 gm. raw veg.*[2]	*pct.*	*pct.*	*pct.*	*pct.*
Cabbage strips	3	Ascorbic acid	2	22.0 mg.	....	50	54	8
Spinach without stems	3	Ascorbic acid	1	14.5 mg.	83	40	36	11
Peas	3	Ascorbic acid	2	8.3 mg.	25	18	20	5
Spinach with stems	4	Thiamin	2	60.5 μg.	101	....	83	8
Spinach with stems	4	Riboflavin	2	102 μg.	109.5	....	108	2

[1] Values were based on the content before storage. [2] The average concentration per milliliter can be calculated from the average volumes of drained liquids given for the weights of the raw vegetables cooked in Tables 1 and 2.

For the liquid from spinach there was no change in thiamin on storage for two days but a loss of about one-fifth on boiling 15 minutes. This treatment had no effect on riboflavin.

Institutional Versus Household Method: From statistical analyses of the results (Table 6) on the retentions of the three vitamins in cabbage wedges and in spinach with stems, cooked both by an institutional and by a household method, the following conclusions can be drawn:

Cabbage wedges and spinach with stems cooked in large quantities in a steam-jacketed kettle showed a greater retention of riboflavin than when cooked in small quantities in an open saucepan but no significant difference in retentions of the other two vitamins. However, if the vegetables are considered as they might be eaten, institutional cabbage held over steam for one and one-half hours was found to be considerably lower in ascorbic acid, but no lower in the other two vitamins, than that freshly cooked in

small quantities in an open saucepan. Institutional spinach that was held showed no difference in ascorbic acid and thiamin retention from the small-scale product but was higher in riboflavin.

TABLE 6

Vitamin Retention [1] *in Vegetables Cooked in Institutional Steam-Jacketed Kettle and in Household Open Saucepan* [2]

Vitamin	Method of cooking cabbage wedges			Method of cooking spinach with stems		
	Household	Institutional		Household	Institutional	
	Freshly cooked	Freshly cooked	After holding [3]	Freshly cooked	Freshly cooked	After holding [3]
	pct.	pct.	pct.	pct.	pct.	pct.
Ascorbic acid	45	51	25	25	33	20
Thiamin	43	46	40	38	50	46
Riboflavin	42	58	46	37	53	51.5

[1] Values were based on the content of the raw vegetable.　[2] Average of four experiments.
[3] The vegetable was held one and one-half hours over steam.

The range of percentage retentions of ascorbic acid for the vegetables cooked in an institution was in keeping with those in the literature. No values for thiamin and riboflavin were found for cabbage, but published figures for spinach do not extend quite as low as some of those making up the average reported in this study. When the vegetables were held over steam, the additional losses of ascorbic acid were statistically significant; percentage retention figures of this vitamin for cabbage were in the lower end of the range and for spinach within the range given in the literature. The changes in thiamin and riboflavin during holding of cabbage and spinach were not significant. For the thiamin in spinach, the average retention here reported after holding one and one-half hours is decidedly higher than the 20-per cent figure of Nagel and Harris (1943) after holding three hours.

Effect of Method of Cutting Vegetables: A comparison of the results included (Fig. 1 and Table 6) showed that cabbage cut in wedges retained more ascorbic acid than that cut in strips when both were cooked in the open saucepan; no statistically significant difference was found for the other two vitamins. Whether spinach was prepared with or without stems made no significant difference for any of the three vitamins in the drained cooked vegetable. In the cooking liquid from spinach, however, the average percentage retentions of thiamin (55 per cent) and riboflavin (52 per cent) when the stems were left on were significantly greater than the 38.5 and 38 per cent, respectively, found in those experiments in which the stems were not included (Fig. 1). No determinations were made for ascorbic acid in the liquid from spinach with stems.

SUMMARY

The ascorbic acid, thiamin, and riboflavin retentions and the acceptability ratings of cabbage strips, spinach without stems, and peas each cooked by four household methods—the open-saucepan, the tightly covered saucepan, the steamer, and the pressure-saucepan—indicated that for green

cabbage and peas the pressure saucepan gave the best product and that for spinach both the steamer and the pressure saucepan were the most satisfactory, when all factors tested were considered simultaneously.

The cooking liquid of these vegetables was stored in a refrigerator for one or two days, and the loss in ascorbic acid was found to be considerable. When the liquid was brought to a boil, one-half to four-fifths of the original ascorbic acid was lost. On boiling 15 minutes no further changes were observed. In the liquid from spinach the only effect on thiamin or riboflavin which occurred under the same conditions was the loss of one-fifth of the thiamin on boiling 15 minutes.

Cabbage wedges and spinach with the stems left on, cooked in large quantities in a steam-jacketed kettle, had approximately the same percentage retention of ascorbic acid and thiamin but more riboflavin than when cooked in small quantities in an open saucepan. Cabbage cooked in an institution and held one and one-half hours over steam, however, was considerably lower in ascorbic acid than the freshly cooked household product but no lower in the other two vitamins. Spinach cooked in an institution and held over steam lost less than a statistically significant amount of ascorbic acid and none of its thiamin or riboflavin; hence, it was still equal to the freshly cooked, small-scale product in ascorbic acid and thiamin and still higher in riboflavin.

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ASCORBIC ACID CONTENT OF STRAWBERRIES

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Strawberries are recognized as being one of the relatively rich sources of vitamin C, and during their season they are an important source of supply for this vitamin. Mayfield and Richardson (1943) reported that frozen and preserved strawberries retained a considerable part of their vitamin content, and these products may be used throughout the year. Numerous workers have reported on the vitamin C content of strawberries. One of the characteristics of the results reported in the literature is the wide variation in amounts of ascorbic acid reported by the different workers, and by the same workers with different varieties and under different conditions. Hansen and Waldo (1944) reported mean values from 52 to 107 mg. per 100 grams fresh weight for 39 different varieties and selections. Burrell and Ebright (1940) reported the ascorbic acid content of the Clermont variety as being 142 mg. per 100 grams, while Slate and Robinson (1946) reported 50 mg. per 100 grams for the same variety. Kirk and Tressler (1941) reported variations of over 100 per cent for samples taken on different days. Satterfield and Yarbrough (1940) reported as much as 63 per cent more ascorbic acid in the high sample over the low, in 10 replicates of the same variety. Olliver (1938) found a 20-per cent variation in ripe berries of the Jucunda variety. Burkhart and Lineberry (1942) found that while it was possible by careful selection to obtain samples that checked quite closely in ascorbic acid content, commercially packed berries of the Klondike variety varied 22 per cent within the same quart basket and 44 per cent between berries taken from different fields. Schuphan (1942) found that four early varieties harvested after several days of bright sunny weather contained from 66 to 100 mg. per 100 grams, while four late varieties harvested after nearly a week of cool, rainy weather contained from 35 to 50 mg. From these results he concluded that bright sunny weather favored high ascorbic acid content, while cool, wet weather resulted in low values; however, the use of different varieties may have been in part responsible for these differences. Hansen and Waldo (1944) reported higher ascorbic acid values following periods of sunshine than following cloudy weather. McCrory (1946) reported that berries exposed to the sun contained 10 per cent more ascorbic acid than did berries of the same plants shaded by the foliage. The berries exposed to the sun and ripening on sunny days contained 10 per cent more ascorbic acid than did similarly exposed berries ripening on cloudy days, while berries shaded by the leaves showed little change between cloudy and sunny days. He also reported that everbearing varieties grown during the long, warm days

of intense light of early June averaged 34 per cent more ascorbic acid than did the same varieties taken in late September. Burkhart and Lineberry (1942) found that berries ripening in the sun contained eight per cent more ascorbic acid than those shaded by the leaves of the plant and 29 per cent more than those ripening under the dense shade of a strawberry cup. Hansen and Waldo (1944) shaded berries as well as both berries and plants. The unshaded berries contained 13 per cent more ascorbic acid than did the shaded ones and 68 per cent more than where plants and berries were shaded.

TABLE 1

Ascorbic Acid Content of Strawberry Varieties Grown in 1942

Variety	Times sampled	Range	Average
Grown at Willard, N. C.		*mg./100 gm.*	*mg./100 gm.*
Fairmore	6	60-92	76
Klonmore	6	47-72	58
Massey	5	45-62	54
Massey x Fairmore (6 selections)	1	46-90	64
Grown at Beltsville, Md.			
Catskill	1	....	81
Fairmore	1	....	76
Starbright	1	....	71
Fairpeake	1	....	70
Suwannee	1	....	67
Ridgeley	1	....	66
Tennessee Shipper	1	....	64
Dunlap	1	....	64
Massey	1	....	58
Maytime	1	....	54
Pathfinder	1	....	51
Aroma	1	....	51
Midland	1	....	50
Missionary	1	....	48
Dorsett	1	....	45
Blakemore	1	....	42
US 3205 (Aberdeen x Fairfax)	1	....	53

Olliver (1938) reported that there was an increase in ascorbic acid from the green to the "nearly red" stage, and that it then remained about constant until the berries were overripe, when there was a slight decrease. She also found that the outer part of the ripe berry contained 86 per cent more ascorbic acid than the inner part. Burkhart and Lineberry (1942) reported a 95-per cent increase from the green to the ripe stage, but only from 10 to 23 per cent more ascorbic acid in the outer cortex than in the inner fleshy pith region.

From the results reported it is evident that the vitamin content of strawberries varies widely, not only between varieties but within varieties as well. Both environmental and hereditary factors play an important part in these variations. Since such wide variations occur under apparently normal growing conditions, it seemed likely that to obtain reliable data both from a nutritional standpoint and an adequate comparison of vari-
eties, more samples would need to be taken than have usually been used in

previous studies. This study was therefore undertaken with the idea that the samples selected would be representative of the variety and that sufficient samples would be analyzed to permit a statistical treatment of the data. Emphasis was placed on testing the varieties that form the bulk of the crop grown in the United States. The effects of shading the plants or the berries, the stage of maturity, and the effect of ripening berries off the plants also received consideration.

PRELIMINARY WORK IN 1942 AND 1944

Following the work reported by Burkhart and Lineberry (1942), Lineberry made a survey of selections and varieties being grown in the cooperative breeding work of the U. S. Department of Agriculture and the North Carolina Agricultural Experiment Station at Willard, N. C., and of certain varieties grown at Beltsville, Md.; results are shown (Table 1).

In 1944 more extensive tests of varieties and selections grown in a single field at Beltsville, Md., were made by the authors. Care was used to pick berries at the same stage of ripeness as indicated by appearance, and the samples were harvested near the same time each morning beginning shortly after eight o'clock. Eight successive pickings were made and in so far as possible five pickings of each variety were tested. On May 22 parts of rows of Blakemore were shaded with a single and with a double thickness of cheesecloth, which reduced the light intensity at midday to 51 and 31 per cent, respectively, of normal daylight. Samples were taken on May 26 and were continued for five successive pickings.

The average ascorbic acid content of nine early, five medium early, and 10 late varieties, the effect of shading on the ascorbic acid content, and pertinent weather data are given (Table 2). The ascorbic acid content of the nine early varieties dropped rapidly at first, rose to a high point on May 31, and then dropped again. The five medium-early varieties showed similar but smaller fluctuations and reached their maximum on June 5, after sampling of the early varieties had been discontinued. The average of the late varieties showed less variation and reached their maximum on June 5 and 7. The effects of shading were not evident at four days but were at seven days. Shading for 14 days, when the tests were terminated, resulted in a 41- and a 53-per cent decrease, respectively, for the lighter-shaded and the heavier-shaded plants.

Ascorbic acid contents of the individual varieties and selections tested in 1944 are shown (Table 3); the first part lists those sampled on four or more picking days, while the second part lists those from which fewer samples were taken. Of all the varieties and selections tested, Aberdeen was consistently the lowest in vitamin C. Maytime, Pathfinder, Dorsett, Missionary, and Blakemore contained less than the average; Tennessee Shipper, Fairfax, Midland, Chesapeake, and Massey were intermediate; and Tennessee Beauty, Catskill, Fairpeake, and Gandy had the highest amounts for the varieties tested. Of the selections tested, U. S. 2153, with 90 mg., was higher than any of the named varieties, and U. S. 2719 and U. S. 3296 surpassed Tennessee Beauty and Catskill in vitamin C content. The lowest selection tested was U. S. 2291, which contained 40 mg., only slightly more than Aberdeen.

TABLE 2

Ascorbic Acid Content of Strawberries, With Temperature and Light Energy Records for Periods Preceding Each Sampling, Beltsville, Md., 1944

Record	May 22	May 26	May 29	May 31	June 2	June 5	June 7	June 9
Ascorbic acid (mg. per 100 gm.)								
Of 9 early varieties.............	61	51	54	68	63	...	...	...
Of 5 medium-early varieties......	...	57	56	61	57	62	...	...
Of 10 late varieties............	...	...	...	...	60	63	63	60
Of Blakemore—no shade..........	...	49	54	52	55	58	...	...
Of Blakemore—single shade......	...	50	41	37	42	41	...	...
Of Blakemore—double shade......	...	50	44	41	40	38	...	...
Light energy [1] (per cent)								
For 3 preceding days...........	52	38	65	94	88	75	63	93
For 1 preceding day............	59	17	85	97	69	39	86	100
Temperature (°F.)								
Average, 3 preceding nights, 8 p.m.-8 a.m...	61	62	61	55	61	64	58	53
Average, 1 preceding night......	65	63	54	55	65	57	65	47
Average, 3 preceding days, 8 a.m.-8 p.m...	65	69	78	80	81	77	69	73
Average, 1 day preceding........	71	64	82	76	83	62	79	71

[1] In percentage of highest light energy of any day of this period.

TABLE 3

Ascorbic Acid Content of Strawberry Varieties Grown and Sampled at Beltsville, Md., 1944

Varieties	Parentage [1]	Ascorbic acid content (mg./100 gm.) when sampled on								Average
		May 22	May 26	May 29	May 31	June 2	June 5	June 7	June 9	
PART I. REGULAR SAMPLING										
Early										
Blakemore...........	Missionary x Howard 17	52	49	54	52	55	58	...	...	53
Missionary...........	Chance seedling	54	43	46	62	56	...	...	...	52
Pathfinder...........	Aberdeen x Howard 17	58	41	42	59	42	...	...	...	48
Maytime............	Missionary x Fairfax	56	43	37	55	51	...	...	...	48
US 3380............	US 2259 x Maytime	82	66	61	73	75	...	...	...	71
US 3230............	US 2259 x Maytime	71	52	66	82	71	...	...	...	68
US 3407............	US 2053 x Maytime	65	53	57	69	83	...	...	...	65
US 3318............	US 2053 x Maytime	65	55	58	70	62	...	...	...	62
US 3224............	US 2053 x Maytime	47	60	61	66	68	...	...	...	60
Early and midseason										
Midland............	Howard 17 x Redheart	...	56	65	58	64	66	...	...	62
Tennessee Shipper......	Blakemore x Missionary	...	52	65	66	58	60	...	...	60
US 2719............	US 1516 x US 2115	...	72	65	76	79	74	...	80	79
US 3205............	Fairfax x Aberdeen	...	51	43	52	41	59	56	62	52
US 2821............	Dorsett x US 1021	...	55	44	54	44	52	...	...	50
Late										
Fairpeake..........	Chesapeake x Fairfax	...	...	...	83	79	75	86	81	81
Tennessee Beauty......	Howard 17 x Missionary	...	...	...	72	62	83	75	72	73
Massey.............	Blakemore x US 634	...	...	...	69	69	72	65	59	67
Chesapeake.........	Chance seedling	...	...	...	...	71	65	60	57	63
Aberdeen...........	Chance seedling	...	...	...	33	33	35	38	34	35
US 2153............	US 261 x Redheart	...	...	...	...	81	89	102	86	90
US 3216............	US 2100 x Dresden	...	...	...	...	55	57	57	54	56
US 3283............	US 2100 x NC 866	...	...	...	...	47	53	58	65	56
US 3257............	Rockhill selfed	...	...	...	...	58	46	55	51	52
US 3422............	Blakemore x Fairmore	...	...	...	...	44	52	50	40	47
Average...........		61	53	55	64	60	63	64	62	60

PART II. LESS FREQUENT SAMPLING

Variety	Parentage									
Gandy	Chance seedling							82	88	85
Catskill	Marshall x Howard 17				70	79	76		59	75
Aroma	Seedling of Cumberland Triumph							70		65
Fragaria virginiana 27	Chance seedling								64	64
Fairfax	Unknown				62	65	58			61
Bedstar	Chesapeake x Fairfax						58		58	58
Dorsett	Howard 17 x Royal Sovereign				51	50	46			49
US 3296	Blakemore x US 2648					80	73			76
US 3417	Maytime x Dresden					73	74			74
US 3226	2259 x Maytime	74	69		83			72		74
US 3211	Dorsett x US 2621							73		73
US 2274	Chesapeake x Redheart									73
US 2183	Missionary x Howard 17			66	72	71	80			72
US 3225	US 2259 x Maytime	74	69							71
US 3414	Catskill x Fairfax					80	60			70
US 2829	Oregon 45 x Fairfax				67	79	62	59	67	67
US 2236	Chesapeake x Fairfax									67
US 2827	Dorsett x NC 367			62	79	60	61			66
US 2407	Ettersburg 326 x Fairfax								58	58
US 2650	Ettersburg 904 x Southland								55	55
US 3243	US 2100 x NC 866					57	55		53	54
US 3409	Blakemore x US 2648					47	58			52
US 3366	Aberdeen x US 2648						50	50	52	50
US 3249	US 2100 x Dresden							46	42	44
US 2291	US 261 x Fairfax			40						40

[1] Parentage of selections referred to but not given in the table:

US 261—Howard Supreme x Klondike	US 1516—US 652 x Fairfax	US 2648—Ettersburg 904 x Fairfax
US 426—Marshall x Rockhill	US 1535—US 652 x Fairfax	NC 225—US 652 x Blakemore
US 542—Portia x Kalicene	US 2053—Howard 17 x *F. ovalis*	NC 302—Blakemore x Fairfax
US 652—Red Sugar x Howard 17	US 2100—Chesapeake x Redheart	NC 367—Blakemore x Fairfax
US 634—Royal Sovereign x Howard 17	US 2115—Chesapeake x Fairfax	NC 640—US 778 x Fairfax
US 778—Missionary x Howard 17	US 2259—US 426 x US 542	NC 866—Blakemore x NC 302
US 1021—Kalicene x Howard 17	US 2621—US 1535 x Fairfax	Oreg. 45—Ettersburg 121 x Marshall

1946 STUDIES

In 1946 the strawberries used were part of a collection grown in a single field in the strawberry production and breeding investigations at the Plant Industry Station, Beltsville, Md. The berries were harvested in the forenoon beginning shortly after eight o'clock and continuing until the samples for that day were finished, usually before noon. The samples were analyzed soon after harvest and no variety was ever held more than three or four hours except in the holding experiments. Four quarts of field-run berries were generally harvested for each sample, and from these four quarts about two quarts of representative berries were selected for analysis. In some cases, where the supply of berries was limited, a smaller amount was used. Each individual sample consisted of 25 grams of tissue taken from at least five berries split lengthwise. In most cases more than five halves were required and additional berries were sampled to provide the necessary amount. In a few cases, however, five half-berries exceeded 25 grams in weight and smaller sections were then used. The method of determination was that of Loeffler and Ponting (1942), using 200 ml. of 0.4 per cent oxalic acid as suggested by Ponting (1943). The ascorbic acid content of the extract was determined in an Evelyn photoelectric colorimeter and samples were compensated for color owing to plant pigments. Ten samples of each variety, selection, or treatment were taken, and the amount reported is the average of the 10 samples. All results are reported on a fresh-weight basis.

RESULTS AND DISCUSSION

The mean ascorbic acid values of 44 varieties and numbered selections are given (Table 4). Most of these were sampled twice in order to obtain information on the seasonal trend in vitamin content during the harvest season. These samplings were intended to represent early- and late-season harvest for the variety and in most cases were four to eight days apart. The results from both of these periods are given in the table. The average ascorbic acid content for all varieties was 63.1 and 61.1 mg. per 100 grams, respectively, for the early and the late harvests, a difference not statistically significant. Twenty-four varieties averaged 6.2 mg. per 100 grams higher at the first picking, while 15 varieties averaged 4.6 mg. higher at the later picking. Sixteen of these differences are statistically significant. Instead of showing a seasonal trend, this indicates that other factors were more important than season in controlling the ascorbic acid content. The varieties ranged from 88.9 to 38.9 mg. per 100 grams.

In addition to the early- and the late-picked lots (Table 4), more frequent harvests were made with the Blakemore and the Fairpeake varieties. The Blakemore was sampled eight times during the season and the Fairpeake six times (Table 5). In the case of the Blakemore variety there was, in general, a decrease in ascorbic acid content as the season advanced but not a significant change from one sampling date until the next except at the last sampling when there was a sharp and significant increase. Between June 6 and June 11 the ascorbic acid content increased from 50.7 to 67.1 mg. per 100 grams, an increase of 32 per cent, while a 13-per cent decrease occurred from May 24 to June 6. The Fairpeake increased sig-

TABLE 4

Ascorbic Acid Content of Strawberry Varieties and Selections,
Beltsville, Md., 1946

Variety	Early picking		Late picking		Difference between pickings [1]	Average	Rank
	Date sampled	Ascorbic acid	Date sampled	Ascorbic acid			
		mg./ 100 gm.		mg./ 100 gm.	mg.	mg.	
Aberdeen.................	June 5	40.2	June 10	42.9	2.7	41.6	43
Aroma......................	....	......	5	50.7	......	50.7	41
Blakemore..............	May 30	59.7	6	50.7	— 9.0[2]	55.2	35
Brightmore.............	30	61.0	7	59.2	— 2.8	60.1	25
Chesapeake............	....	......	6	69.2	......	69.2	11
Donner...................	30	56.8	10	62.0	5.2[2]	59.4	28
Dorsett...................	24	65.8	May 30	60.4	— 5.4[3]	63.1	22
Dunlap...................	31	72.7	June 6	59.3	—13.4[2]	66.0	17
Fairfax...................	31	64.4	6	66.6	2.2	65.5	18
Fairpeake..............	June 6	69.7	10	79.3	9.6[2]	74.5	3
Gandy...................	7	67.7	10	70.5	2.8	69.1	12
Howard 17............	May 29	59.7	5	66.7	7.0[2]	63.2	21
Joe.......................	31	71.8	6	69.0	— 2.8	70.4	7
Klondike...............	31	65.3	5	67.5	2.2	66.4	14
Konvoy.................	June 5	56.4	11	60.8	4.4	58.6	31
Marshall...............	....	......	11	77.4	......	77.4	2
Massey.................	May 31	63.0	6	53.5	— 9.5[2]	58.3	32
Maytime...............	24	64.1	5	54.4	— 9.7[2]	59.3	29
Midland................	30	60.2	5	58.3	— 1.9	59.3	30
Missionary............	29	48.5	5	45.7	— 2.8	47.1	42
Redstar.................	June 10	70.1	14	68.4	— 1.7	69.3	10
Robinson...............	6	68.1	10	74.0	5.9	71.1	4
Shasta...................	3	58.9	7	52.4	— 6.5	55.7	34
Sparkle.................	May 31	59.6	7	56.0	— 3.6	57.8	33
Suwannee.............	29	71.0	5	69.7	— 1.3	70.4	8
Temple.................	29	66.4	6	66.8	0.4	66.6	13
Tenn. Beauty........	30	64.6	12	62.6	— 2.0	63.6	20
Tenn. Shipper.......	June 6	67.0	12	74.5	7.5[3]	70.8	6
Calif. 467.............	3	58.0	11	61.4	3.4	59.7	27
Calif. 590.............	3	67.4	11	73.2	5.8	70.3	9
Md. 430................	....	......	11	60.4	......	60.4	24
NC 1039...............	10	67.0	12	65.3	— 1.7	66.2	16
NC 1162...............	5	53.2	10	52.2	— 1.0	52.7	37
US 2153...............	3	93.2	7	84.6	— 8.6[2]	88.9	1
US 2700...............	May 24	84.7	6	57.1	—27.6[2]	70.9	5
US 3203...............	June 3	39.5	7	38.3	— 1.2	38.9	44
US 3205...............	May 24	63.6	May 30	56.4	— 7.2[3]	60.0	26
US 3283...............	30	55.6	June 7	53.5	— 2.1	54.6	36
US 3289...............	June 3	60.8	7	65.1	4.3[2]	63.0	23
US 3358...............	....	......	14	50.8	......	50.8	40
US 3366...............	3	53.9	7	50.1	— 3.8	52.0	38
US 3417...............	May 24	71.2	7	59.1	—12.1[2]	65.2	19
US 3500...............	30	55.9	7	45.9	—10.0[2]	50.9	39
US 3504-2-4.........	June 5	63.3	11	69.3	6.0	66.3	15

[1] Difference required between varieties for significance at five-per cent level: early pick 4.4, late pick, 4.5; at one-per cent level: early pick 5.7, late pick 5.9. [2] Significant difference between pickings at one-per cent level. [3] Significant difference between pickings at five-per cent level.

nificantly (16 per cent) between June 7 and 10, and then remained comparatively high until the last sample was taken on June 14.

That both of these varieties should increase appreciably at about the same time appears significant; also the fact that of the five varieties (Table 4) that showed a significant increase at the second picking, three were taken on June 10, 11, or 12. Of the 11 varieties that showed a significant decrease at the second picking, nine were taken on June 5, 6, and 7. From these results it would appear that climatic or other conditions were unfavorable for high ascorbic acid production on and immediately preceding June 5, 6, and 7, but that conditions had improved appreciably by June 10, 11, and 12. That the increase at this time is primarily due to

TABLE 5

Variation [1] *in Ascorbic Acid Content of Blakemore and Fairpeake Strawberries During Harvest Season, Beltsville, Md., 1946*

Date	Blakemore	Fairpeake
	mg./100 gm.	*mg./100 gm.*
May 24	58.4	......
29	56.6	......
30	59.7	......
31	55.8	......
June 3	54.2	71.2
5	54.3	......
6	50.7	69.7
7	......	68.1
10	......	79.3
11	67.1	......
12	......	77.6
14	......	75.9

[1] Difference required for significance at five-per cent level: 4.5 for Blakemore, 5 1 for Fairpeake; at one-per cent level: 6.0 for Blakemore, 6.8 for Fairpeake.

more sunlight is indicated by the fact that the shaded berries did not respond similarly but instead continued to decline in ascorbic acid. Climatological data published by the U. S. Weather Bureau (1946) for this period for Washington, D. C., are given (Table 6). Assuming that light intensity is the principal environmental factor involved in ascorbic acid production and that the percentage of possible sunshine available is a legitimate measure of this, it appears that light intensity for approximately one week prior to harvest is operative on the ascorbic acid production, as the pattern of the average percentage of sunshine for the week previous to harvest more nearly conforms to the ascorbic acid pattern than is the case for shorter periods. Additional evidence that light exerts an influence for several days before the berries are ripe is furnished by the shading experiments in which shading for four days in 1944 and for six days in 1946 caused no reduction in the ascorbic acid content, while seven days and eight days or longer, in these two years, caused a reduction in the shaded lots. It seems more likely, however, that an interrelationship exists between different climatic factors and ascorbic acid production rather than a simple sunshine and ascorbic acid relationship, even though

the light factor may be, and probably is, the dominant one. The normal amount of sunshine for May and June in this area is 61 per cent. The mean for May 15 through June 14, 1946, was 62 per cent, and thus was near normal.

TABLE 6

Climatological Data, Washington, D. C., May 15 to June 14, 1946

Date	Temperature			Departure from normal	Precipitation	Sunshine[1] (possible)	Character of day
	Max.	Min.	Mean				
	°F.	°F.	°F.	°F.	in.	pct.	
May 15	68	54	61	− 2	T	18	Cloudy
16	82	63	72	8	0.04	23	Cloudy
17	85	67	76	12	T	44	Cloudy
18	78	61	70	6	1.17	32	Cloudy
19	80	59	70	5	0.00	86	Clear
20	81	62	72	7	0.17	32	Cloudy
21	76	63	70	5	0.24	53	Partly cloudy
22	76	55	66	0	0.00	100	Clear
23	79	51	65	− 1	0.00	100	Clear
24	79	52	66	0	0.00	99	Partly cloudy
25	89	65	77	11	0.00	100	Clear
26	80	67	74	7	0.95	17	Cloudy
27	84	62	73	6	0.95	51	Cloudy
28	66	53	60	− 7	0.09	0	Cloudy
29	72	50	61	− 7	0.00	100	Clear
30	85	52	68	0	0.00	100	Clear
31	88	59	74	6	0.00	87	Partly cloudy
June 1	87	68	78	10	0.01	71	Partly cloudy
2	70	55	62	− 7	0.78	44	Cloudy
3	76	57	66	− 3	T	81	Partly cloudy
4	71	54	62	− 7	0.27	50	Partly cloudy
5	72	52	62	− 8	0.00	95	Clear
6	76	51	64	− 6	T	60	Partly cloudy
7	88	57	72	2	0.00	69	Partly cloudy
8	93	68	80	10	0.00	85	Clear
9	87	66	76	5	0.00	100	Clear
10	75	55	65	− 6	0.00	92	Partly cloudy
11	90	60	75	4	0.00	78	Partly cloudy
12	83	72	78	6	T	25	Cloudy
13	85	71	78	6	T	42	Cloudy
14	75	59	67	− 5	0.03	9	Cloudy

[1] Sunshine normal for May and June—61 per cent; mean for May 15 to June 14, 1946—62 per cent.

To study the effect of shading on the ascorbic acid content, Blakemore and Fairpeake strawberry plants were covered with one thickness (light shade) and four thicknesses (heavy shade) of cheesecloth on May 23, 1946, and the ascorbic acid content of the berries from the shaded plants was compared with that of fruit grown in the open. The shaded plants were completely covered, the cheesecloth reaching to the ground on all sides. Light readings made with a light-meter showed that the light intensity was reduced to approximately 64 and 43 per cent of full sunlight at midday under the light and the heavy shades, respectively. Another phase of the shading experiment was the effect of covering the berries only. This was done by enclosing the berries in small brown manila bags nine days

before they were ripe. Berries of comparable maturity, tagged but left uncovered, ripened on the same date as those covered; results of the shading experiments are given (Table 7).

TABLE 7

Effect of Shading (Begun May 23, 1946) on Ascorbic Acid Content of Strawberries

Date	Normal sunlight	Light shade	Heavy shade	Only berries covered
Blakemore	mg./100 gm.	mg./100 gm.	mg./100 gm.	mg./100 gm.
May 29	56.6	61.9[1,2]	59.0	......
30	59.7	......	......	49.9[1,4]
31	55.8	51.6[1]	52.1[2]	......
June 3	54.2	46.2[2]	45.2[2]	......
11	67.1	45.3[2]	42.1[2]	......
Fairpeake				
June 6	69.7	59.0[2]	60.2[2]	......
10	79.3	60.6[2]	61.4[2]	......
12	77.6	58.2[2]	53.7[2,5]	......
12	76.2[6]	......	......	63.9[2]
14	75.9	56.6[2]	47.2[2,7]	57.0[2,8]

[1] Difference between sunlight and shade significant at five-per cent level. [2] Difference between sunlight and shade significant at one-per cent level. [3] Shaded significantly higher. [4] Five replicates only in this sample. [5] Difference between light and heavy shade significant at five-per cent level. [6] Berries tagged but ripened in open; six replicates only. [7] Difference between light and heavy shade significant at one-per cent level. [8] Three replicates only in this sample.

With one exception samples taken on all dates gave significantly lower values under the shade. Samples of Blakemore taken on May 29, six days after the shade was applied, showed that the lightly shaded berries were significantly higher in ascorbic acid than the unshaded ones; the heavily shaded lot also averaged higher but not significantly so. The reason for this exception is not known. Perhaps the shade had not had sufficient time to exert a controlling influence and other factors may have been more favorable to ascorbic acid production under the cheesecloth than outside during this time. The fact that several days were required before the effect of the shade was reflected in the ascorbic acid content suggests the possibility that the light exerts its maximum influence before the fruit is ripe, possibly during the period when a vitamin C precursor or precursors are being formed, rather than during the formation of the vitamin itself. This hypothesis would be in keeping with the observation reported earlier in this paper, that the average light intensity for one week prior to harvest more nearly conformed to the ascorbic acid pattern than did that for shorter periods. It would also be in agreement with results reported later in this paper in which an indication is given that the ascorbic acid increased in berries held in the laboratory for 24 and 48 hours.

With the Blakemore there was no significant difference in ascorbic acid content between the light and heavy shade, and only on the last two sampling dates was there significant difference in the case of Fairpeake. Thus, while the effect of the light shade was apparent in ascorbic acid production within eight days, it required 20 days for the heavier shade to show additional effect.

TABLE 8

Ascorbic Acid Content of Strawberry Varieties and Selections Adjusted for Climatic Variations During Harvest Season, Beltsville, Md., 1946

Variety	Early picking		Late picking		Difference between pickings	Average	Rank
	Date sampled	Ascorbic acid	Date sampled	Ascorbic acid			
		mg./ 100 gm.		*mg./ 100 gm.*	*mg.*	*mg.*	
Aberdeen......................	June 5	43.2	June 10	38.5	— 4.7[1]	40.9	44
Aroma..........................	....	......	5	54.5	......	54.5	36
Blakemore....................	May 30	58.4	6	58.4	0	58.4	31
Brightmore...................	30	59.7	7	61.8	2.1	60.8	25
Chesapeake...................	....	......	6	79.7	......	79.7	2
Donner.........................	30	55.6	10	55.7	0.1	55.7	33
Dorsett.........................	24	65.8	May 30	59.1	— 6.7[2]	62.5	23
Dunlap.........................	31	76.1	June 6	68.3	— 7.8[2]	72.2	10
Fairfax.........................	31	67.4	6	76.7	9.3[2]	72.1	11
Fairpeake.....................	June 6	80.3	10	71.2	— 9.1[2]	75.8	4
Gandy..........................	7	70.7	10	63.3	— 7.4[2]	67.0	15
Howard 17.....................	May 29	61.5	5	71.7	10.2[2]	66.6	17
Joe...............................	31	75.2	6	79.5	4.3[1]	77.4	3
Klondike.......................	31	68.4	5	72.6	4.2	70.5	12
Konvoy.........................	June 5	60.6	11	52.9	— 7.7[1]	56.8	32
Marshall.......................	....	......	11	67.4	......	67.4	14
Massey..........................	May 31	66.0	6	61.6	— 4.4[1]	63.8	20
Maytime........................	24	64.1	5	58.5	—5.6[2]	61.3	24
Midland........................	30	58.9	5	62.7	3.8	60.8	26
Missionary....................	29	49.9	5	49.1	— 0.8	49.5	41
Redstar.........................	June 10	63.0	14	64.2	1.2	63.6	21
Robinson.......................	6	78.4	10	66.5	—11.9[2]	72.5	9
Shasta..........................	3	63.5	7	54.8	— 8.7[2]	63.5	22
Sparkle.........................	May 31	62.4	7	58.5	— 3.9	60.5	27
Suwannee......................	29	73.1	5	74.9	1.8	74.0	6
Temple..........................	29	68.4	6	76.9	8.5[2]	72.7	8
Tenn. Beauty.................	30	63.2	12	57.4	— 5.8	60.3	28
Tenn. Shipper................	June 6	77.2	12	68.3	— 8.9[2]	72.8	7
Calif. 467......................	3	62.5	11	53.4	— 9.1[2]	53.0	37
Calif. 590......................	3	72.6	11	63.7	— 8.9[1]	68.2	13
Md. 430........................	....	......	11	52.6	......	52.6	38
NC 1039........................	10	60.2	12	59.9	— 0.3	60.1	29
NC 1162........................	5	57.2	10	46.9	—10.3[2]	52.1	39
US 2153........................	3	100.4	7	88.4	—12.0[2]	94.4	1
US 2700........................	May 24	84.7	6	65.8	—18.9[2]	75.3	5
US 3203........................	June 3	42.6	7	40.0	— 2.6[1]	41.3	43
US 3205........................	May 24	63.6	May 30	55.2	— 8.4[2]	59.4	30
US 3283........................	30	54.4	June 7	55.9	1.5	55.2	34
US 3289........................	June 3	65.5	7	68.0	2.5	66.8	16
US 3358........................	....	......	14	47.6	......	47.6	42
US 3366........................	3	58.1	7	52.3	—5.8[1]	55.2	35
US 3417........................	May 24	71.2	7	61.8	— 9.4[2]	66.5	18
US 3500........................	30	54.7	7	48.0	— 6.7[2]	51.4	40
US 3504-2-4...................	June 5	68.1	11	60.3	— 7.8[1]	64.2	19

[1] Significant difference between pickings at five-per cent level.　[2] Significant difference between pickings at one-per cent level.

Since climatic conditions do greatly affect the ascorbic acid content, one might question the relative values and accuracy of varietal comparisons made on different dates, especially in sections of the country where climatic conditions vary widely. In this part of the country it is seldom that uniform conditions prevail throughout the strawberry harvest season. As a possible means of correcting for this effect, we arbitrarily chose the ascorbic acid content of the Blakemore variety on May 24 as representing 100. Any increase or decrease in this variety on succeeding dates was calculated as a percentage and added to or subtracted from the determined value so as to bring it to the 100 basis. A like percentage of increase or decrease was applied to other varieties picked on that date. On June 3 both Blakemore and Fairpeake were harvested. Fairpeake harvested on this date was adjusted as stated above, and this variety after adjustment was used thereafter as the check variety if Blakemore was not available. It is here assumed that the climatic effects are the same on different varieties and that hereditary effects are constant. While the assumption that the climatic effects are the same on all varieties probably is not strictly true, the method does take into consideration atmospheric conditions that heretofore have been largely ignored and their effects incorrectly ascribed to varietal differences.

Adjustments as described above have been applied (Table 8) to the data in Table 4. It will be noted that while in general the high varieties are still high and the low ones are low, the relative position of many varieties is changed, some quite considerably. Marshall, which was second from the top (as determined on one date only), dropped to 14th in the adjusted list, while Chesapeake (also one date only) moved from 11th to second place. In general, the effect was to raise slightly the whole list. Climatic conditions on and preceding May 24 seem to have been more favorable for ascorbic acid production than the average for the season. In fact, the average amount of sunshine for the week ending May 24 was 71 per cent in comparison with 62 per cent for the whole harvest period.

Another effect of the adjustment for climatic conditions (Table 8) is that 26 of the varieties now show lower ascorbic acid values at the second picking than at the first, and 22 of these differences are statistically significant. Twelve varieties show an increase at the second picking, but only four of these are significantly higher. The range between varieties remains about the same, 88.9 to 38.9 mg. per 100 grams as determined and 94.4 to 40.9 mg. after adjustment. For other workers who may be interested in using the above method for adjusting for climatic conditions, it is suggested that, in order to facilitate comparison of varieties tested in different parts of the country, Blakemore be used as the check variety when available.

Slate and Robinson (1946) reported that small berries tended to have a higher concentration of vitamin C than large berries. Olliver (1938) and Burkhart and Lineberry (1942) have reported that the outside of the berry was higher in ascorbic acid than the inside. This also would indicate that smaller berries should be higher in ascorbic acid than the larger ones, since the smaller ones have a proportionally larger exposed area. The average size of the berries tends to decrease as the season advances, and

other things being equal, one might expect the late-picked fruit to be higher in ascorbic acid than the early-picked. This is contrary to our results, where the majority of the varieties decreased at the later pickings, which would indicate that climatic conditions were more important in determining the ascorbic acid content than size alone.

To study the effect of degree of maturity on the ascorbic acid content, a series each of Blakemore and Fairpeake berries was picked at different stages of maturity designated as white, pink, half-red, and ripe; results from these tests are given (Table 9). There was a consistent increase in ascorbic acid as maturity advanced. This agrees with the results of Olliver (1938) for the Jucunda variety.

TABLE 9

Effect of Maturity on Ascorbic Acid Content of Strawberries

Stage of maturity	Blakemore [1]	Fairpeake [1]
	mg./100 gm.	*mg./100 gm.*
White..	37.7	63.8
Pink...	40.2	71.5
Half-red..	45.8	77.5
Ripe...	56.6	82.7
Half-red, held 1 day..	49.0	......
Half-red, held 2 days...	......	80.4

[1] Difference required for significance at five-per cent level: 4.1 for Blakemore, 5.9 for Fairpeake; at one-per cent level: 5.5 for Blakemore, 7.9 for Fairpeake.

Samples of berries that were classed as half-red and that are sometimes held until fully colored before marketing were divided into two parts. One part was tested immediately and the other part was held in the laboratory one day (Blakemore) or two days (Fairpeake) until fully red (Table 9). While the berries held in the laboratory did not contain as much ascorbic acid as those fully ripened on the plant, there was an increase over the comparable half-red fruit determined earlier. While the number of samples taken did not show a significant difference statistically, it is possible that had a larger number of samples been taken they would have shown a significant increase and furnished additional proof that ascorbic acid is formed in the berry. Other evidence in support of this hypothesis is that the berry tips showed a consistently and significantly higher ascorbic acid content than did the stem ends. Thirteen pairs of samples averaged 65.1 for the tips and 60.3 mg. per 100 grams for the stem ends, the difference probably being due to shading of the stem end by the calyx.

The leaves of Aberdeen, Blakemore, and Fairpeake, representing varieties of low, medium, and high ascorbic acid content, were analyzed and the results are given (Table 10). From these results it is evident that (1) the leaves are much higher in ascorbic acid than the fruit; (2) there is no direct correlation between the vitamin content of the leaves and that of the fruit [these results confirm the work of Hansen and Waldo (1944)]; (3) the leaves are significantly different in different varieties; and (4) the ascorbic acid content of the leaves changed significantly in two of the varieties within two days even though the weather was cloudy on both days.

In 1944 the varieties of strawberries grown in the United States ranked in acreage about as shown (Table 11). The ascorbic acid content of these varieties is also indicated.

The ascorbic acid content of strawberries is dependent upon the variety and the weather conditions under which they are grown. Prominent varieties grown in the South are Blakemore, Klondike, Klonmore, Massey, and Missionary, which would average about 50 mg. per 100 grams for approximately 50 per cent of the crop of the United States. In the Pacific Northwest Marshall, Corvallis, and Redheart are all high in ascorbic acid, averaging between 75 and 80 mg. for about 12 per cent of the national crop. In the Northeast the ascorbic acid content of the many varieties grown is variable but may average 60 to 65 mg. for 30 per cent of the crop of the United States. The average for the entire crop of the country is probably around 60 mg. per 100 grams.

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TABLE 10

Ascorbic Acid Content of Strawberry Leaves

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Date	Fairpeake [1]	Blakemore [1]	Aberdeen [1]
	mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*
June 12	220.6	291.5	266.0
14	210.1	258.8[2]	238.4[2]

[1]Difference required for significance between varieties at one-per cent level: 21.0 mg.   [2]Difference highly significant between dates.

Of the more important commercial varieties, Redheart, Catskill, and Marshall, and of the less important or new varieties, Corvallis, Gandy, Fairpeake, Fairmore, and Tennessee Beauty, all have high ascorbic acid content. These eight varieties do not have any known common morphological or physiological characteristics that might be associated with high ascorbic acid content. Fairpeake, Marshall, Catskill, and Fairmore are sweet, while Gandy is quite tart. Redheart, Marshall, and Fairmore are dark colored, while Fairpeake has a bright color. Redheart, Fairpeake, and Fairmore are firm fleshed, while Catskill and Marshall are soft berries. Redheart, Corvallis, and Marshall are varieties adapted to the Northwest; Fairmore to the South; and Fairpeake, Gandy, and Tennessee Beauty to the midnortheastern regions. The Beacon variety was reported with 104 mg. at Geneva, N. Y., and the Southland with 93 at Geneva and 49 at Willard, N. C. Several unnamed selections of high ascorbic acid content are listed (Tables 3 and 4).

The same conditions that produce the highest flavor in any variety of strawberry seem also to produce the highest ascorbic acid content. The conditions for best flavor are high light intensity, warm but not too hot days, cool nights, and long daily light periods. High light intensity with moderately warm days and cool nights seems to favor a high ascorbic acid content.

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SUMMARY

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A study was made of the ascorbic acid content of strawberries in which a sufficient number of samples was taken to permit a statistical analysis of the data.

TABLE 11

Ascorbic Acid Content of Strawberry Varieties of the United States

Leading varieties

Rank and name	Per cent of total acreage	Vitamin C content (mg./100 gm.) Beltsville, Md. 1944	Vitamin C content (mg./100 gm.) Beltsville, Md. 1946	Other reports
1—Blakemore	34	53	55	42,[1] 43[5]
2—Howard 17	12	...	63	56,[10] 54[0]
3—Marshall	11	...	77	78,[8] 77,[8] 49[9]
4—Klondike	7	...	66	59,[2] 39,[5] 48,[6]
5—Klonmore	7	...	...	58,[2] 58[8]
6—Aroma	6	65	51	51[1]
7—Missionary	4	52	47	48,[1] 36[5]
8—Catskill	4	75	...	81,[1] 81[9]
9—Fairfax	2	61	66	55,[5] 68,[7] 66,[10] 62[9]
10—Lupton	2	...	...	
11—Redheart	1	...	...	84,[7] 69[9]
12—Beaver	1	...	...	
13—Dorsett	1	49	63	45,[1] 45,[5] 59,[7] 99[9]
14—Massey	1	67	58	58,[1] 56,[2] 54,[6] 56[9]
15—Robinson	1	...	71	70[9]
Others	6	...	...	

Other varieties

Rank and name	Vitamin C content (mg./100 gm.) Beltsville, Md. 1944	Vitamin C content (mg./100 gm.) Beltsville, Md. 1946	Other reports
Chesapeake	63	69	63[7]
Pathfinder	48	...	51,[1] 49,[10] 55[9]
Maytime	48	59	51,[1] 72,[8] 57[9]
Aberdeen	35	42	49,[10] 41[9]
Brightmore	...	60	55[9]
Gandy	85	69	
Dunlap	...	66	64,[1] 79,[3] 64,[4] 64[10]
Fairmore	...	...	76,[1] 78,[2] 65,[5] 68[6]
Gem	...	...	68,[4] 65,[9] 56[10]
Camden	...	...	54[7]
Clermont	...	...	73,[7] 50[9]
Culver	...	...	60,[7] 49[9]
Corvallis	...	...	86,[7] 86,[8] 83[8]
Temple	...	67	63[9]
Other new varieties			
Tenn. Shipner	60	71	64,[1] 75[9]
Tenn. Beauty	73	64	75[9]
Midland	62	59	50,[1] 64[9]
Sparkle	...	58	65[9]
Fairpeake	81	75	70[1]
Suwannee	72	70	67,[1] 68[9]

[1] 1942—Berries grown at Beltsville and shipped to Raleigh, N. C.
[2] 1942—Berries grown at Willard, N. C.
[3] 1940—Berries grown in Montana—Mayfield and Richardson (1943).
[4] 1940—Berries grown in Montana—Mayfield and Richardson (1943).
[5] 1938—Berries grown in North Carolina—Satterfield and Yarbrough (1940).

[6] 1942—Berries grown in North Carolina—Satterfield and Yarbrough (1940).
[7] 1940—Berries grown at Geneva, N. Y.—Kirk and Tressler (1941).
[8] 1943—Berries grown at Corvallis, Ore.—Hansen and Waldo (1944).
[9] 1945—Berries grown at Geneva, N. Y.—Slate and Robinson (1946).
[10] 1946—Berries grown at Brookings, S. D.—McCrory (1946).

Twenty-eight named varieties and 16 numbered selections ranged in ascorbic acid content from 88.9 to 38.9 mg. per 100 grams, with a varietal difference of 4.5 and 5.9 mg. per 100 grams required for significance at the five-per cent and the one-per cent level, respectively.

Of the more important varieties, Marshall, Catskill, Redheart, and Robinson and of the less important or new varieties, Gandy, Fairmore, Corvallis, Tennessee Beauty, Fairpeake, and Suwannee have high ascorbic acid values.

Strawberries grown in the shade contained significantly less ascorbic acid than did those exposed to normal sunlight. For 1944 the unshaded had values 41 and 53 per cent greater than the single-shaded and double-shaded, respectively, and for 1946 the unshaded had increases of 48 and 59 per cent for Blakemore and 34 and 61 per cent for Fairpeake in ascorbic acid content over the lighter-shaded and the heavier-shaded plants, respectively.

A method of adjusting for climatic conditions in varietal tests made on different dates is suggested and applied to the varieties reported.

Half-red berries held for one and for two days until fully red increased in ascorbic acid content but not so much as those ripening on the plant.

Though the ascorbic acid content of strawberries in the United States varies with the variety and climatic conditions, it probably averages around 60 mg. per 100 grams; but this value might be raised by breeding to 80 mg. or more.

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